

THERMAL BIOLOGY AND NEST-SITE SELECTION IN
THE BEE 'HALICTUS RUBICUNDUS' (HYMENOPTERA
: HALICTIDAE)

Simon G. Potts

A Thesis Submitted for the Degree of PhD
at the
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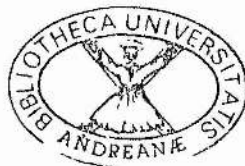
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**Thermal Biology and Nest-site Selection
in the Bee *Halictus rubicundus*
(Hymenoptera: Halictidae)**

Simon G Potts

Thesis submitted for the degree of Doctor of Philosophy,
University of St. Andrews

August 1995



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Declaration

I, Simon G. Potts, hereby certify that this thesis has been composed by myself, and that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

I was admitted to the Faculty of Science of the University of St. Andrews under general ordinance General No. 12, and as a candidate for the degree of Ph.D., on 1 October 1991.

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This thesis is dedicated to my family, Helen, Tory, Geoff and Magsie. My introduction to biology proper began at an early age through the numerous treks made around the Yorkshire Dales with my Grandma. Her almost encyclopaedic knowledge of local natural history astounds me even now; and this in conjunction with the ceaseless flow of newspaper articles, magazines and books have been a constant source inspiration. This is then for her.

He will watch from dawn to gloom
The lake-reflected sun illumine
The yellow bees in the ivy-bloom,
Nor heed nor see, what things they be;
But from these create he can
Forms more real than living man....

Percy Bysshe Shelly (1792 - 1822)

For among Bees and Ants are social systems found
so complex and well-ordered as to invite offhand
a pleasant fable enough: that once upon a time,
or ever a man was born to rob their honeypots,
bees were fully endowed with reason and only lost it
by ordering their life as to dispense with it;
whereby it pined away and perished of disuse.

Robert Bridges (1844 - 1930), The Testament of Beauty

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Thermal Biology and Nest-site Selection in the Bee *Halictus rubicundus*
(Hymenoptera: Halictidae)

Abstract

Aggregations of the ground-nesting bee *Halictus rubicundus* were found at several locations across the UK. The phenology, social organisation, nest architecture and foraging behaviour of this bee were described for the largest of these aggregations (Invergowrie, Scotland). This site was unusual in having an extremely high brood mortality due to the impact of an anthomyiid fly. Nest parasitism was found to be directly density-dependent and it led to a marked decline in nest numbers over the period of this study, indicating the possible forthcoming extinction of the aggregation.

The other sites contained smaller nesting aggregations and the level of parasitism was considerably less. There was a marked variation in size across UK populations and this was explained by a temperature rather than a latitudinal cline.

There was no evidence from field or laboratory studies to suggest that this species is endothermic; *H. rubicundus* is purely a behavioural thermoregulator. The effect of size upon various rates of heat exchange were examined in the laboratory for both sexes, and related to behaviours observed at the nest-site. Thus the microclimatic windows for different activities were established. The abundance of flying individuals at the nest-site was highly predictable from ambient temperature and light intensity; with predictions during a single day being more accurate than those combining several days throughout the season. Furthermore the usefulness of standard operative temperatures in predicting flight activity was assessed. The thoracic temperature of both sexes depended on the prevailing ambient temperature and also on the size of the individual while either basking or flying. Body temperatures increased with both ambient temperature and head width. However when both these predictors were combined into a single model, then size was only a strong predictor at lower temperatures. These findings were used to explain many of the behavioural patterns observed at Invergowrie.

The nest site selection behaviour of females was examined both within and across sites. *H. rubicundus* was able to utilise a range of edaphic and microclimatic conditions when choosing a site to excavate a nest. There were some factors with broad tolerances such as slope and hardness, and others with much narrower limits such as aspect, soil humidity and particle composition. The thermal advantages of having a warm nest meant that the most suitable areas were those with a southern aspect and a slope which

maximised the absorption of solar radiation. Limited areas of substrate with the most desirable characteristics resulted in gregarious nesting ('limited substrate' hypothesis). There was a preference for softer soils, that were easier to dig, within a site with a low overall density; but in much denser aggregations problems of maintaining the structural integrity of a nest lead to the favouring of harder soils. The tendency to nest in close proximity to the natal nest (philopatry) complemented the 'limited substrate' hypothesis in producing an aggregation of nests. The spatial distributions of nests within aggregations were examined using nearest neighbour distance analyses; at low densities, microscale variation in substrate quality produced clumped patterns, whereas at high densities the risk of adjacent nests collapsing into one another forced nest spacing to be more regular.

Findings concerning temperature dependence of nesting and foraging activities, and broader environmental controls on nest-site selection, are considered in relation to key aspects of bee biology: the origins and function of social behaviours, the conservation of, or provision of, nest-sites, and the utility of solitary bees as crop pollinators.

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Chapter 1 Introduction.

1.1 A brief review of insect thermoregulation.

Temperature fundamentally influences all aspects of an insect's physiology and behaviour. At the cellular level the rates of biochemical processes are temperature dependent, and this in turn affects the performance capabilities of locomotion, feeding, growth and reproduction (Casey 1992). The type and range of activities that an insect exhibits will depend upon its body temperature (T_b). For any process or behaviour there will be an optimum (or optimal range) as well as a minimum and maximum at which the insect can function.

Owing to their relatively small size insects show high rates of heat exchange with their environment; therefore unless they are able to thermoregulate, their activity is almost entirely governed by changes in the thermal environment. Thermoregulation implies that variation in the temperature of the body (or part of it) is less than the variation in ambient temperature (T_a) over a range of environmental conditions. In fact many insects have evolved methods to control their T_b within narrow limits independently of T_a , and so are active outside the thermal constraints that would otherwise be limiting.

Behavioural thermoregulation is demonstrated in varying degrees by all insects. The selection of a suitable microclimatic niche within a thermally heterogeneous environment allows an insect to control its T_b to some extent. Insects that depend upon an external heat source for the maintenance of a particular T_b are ectotherms. Direct and reflected solar radiation are the primary avenues for heat gain from external sources although re-radiated long wave radiation from the substrate may be important in some cases. Basking, sun-shade shuttling, posturing, orientation and burrowing are all well documented examples of

behavioural thermoregulation (reviewed by Willmer 1982; Casey 1988; Unwin & Corbet 1991).

In addition to behavioural means for controlling T_b some insects have developed mechanisms for physiological thermoregulation. Endothermy is the ability to elevate T_b using internally generated heat (from muscular activity for example) in order to meet certain heat requirements of the individual. The timing of the muscular endothermy during non-flight activity can be controlled. Specific examples are pre-flight warm-up by shivering in many large insects (Heinrich 1993) and brood incubation in bumblebees (Heinrich 1974).

All insects produce some heat as a by-product of metabolic processes; and their flight muscles are some of the most metabolically active tissues known, and occupy most of the thorax of actively flying species (Kammer & Heinrich 1974). As the muscles are, mechanically, only about 20% efficient, 80% of the energy is dissipated as heat and so active flight can lead to an increase in T_b (Bartholomew 1981). The generation of an elevated T_b above T_a , or temperature excess (T_{ex}), from an internal heat source is obligatory in this case, and so does not strictly qualify as endothermy. Thoracic temperature (T_{th}) regulation in flight has been demonstrated in many insects e.g. the hawkmoth *Hyles lineata* (Heath & Adams 1965) and in bumblebees, *Bombus* spp. (Heinrich 1972, 1975). The main advantage of an elevated T_b is that an insect can remain active at a lower T_a .

Flight muscles are adapted to function with maximum power output at the high temperatures they normally experience during flight (Heinrich 1977). Large bees, moths, dragonflies and beetles that maintain a large T_{ex} during flight are unable to generate enough lift to fly with a T_b near the T_a normally encountered. The characteristic thermal regime of an insects environment will of course influence this to some extent, with tropical insects generally being less restricted than cool

temperate counterparts. In many cases it is therefore necessary to increase T_b prior to flight to ensure that a sufficiently high power output and wing beat frequency can be attained. A T_{th} of $\approx 40^\circ\text{C}$ is necessary before some larger insects choose to fly (Heinrich 1993). Preactivity warm-up is associated with high levels of muscular activity; for most insect groups the thoracic flight muscles are involved in shivering, although the tymbal muscles are used by cicadas prior to singing (Stevens & Josephson 1977).

In flight the thoracic muscles contract alternately to raise and lower the wings; for preflight warm-up, however, the opposing muscles contract antagonistically and in phase. For bumblebees these contractions are synchronous and there is no wing movement; moths, butterflies and dragonflies use opposing phasic contractions slightly asynchronously and this results in low amplitude wing vibrations or 'wing-whirring' (Krogh & Zeuthen 1941). As T_{th} increases the rate of tension rise and fall increases, twitch duration decreases and heat generation increases (Josephson 1981); this is the result of the effect of rising temperature on the CNS (Hanegan and Heath 1970).

Non-shivering thermogenesis has been proposed as a biochemical method for increasing T_{th} in the absence of muscle contractions (Crabtree and Newsholme 1972). It has been suggested that bumblebees use a pair of enzymes to carry out a substrate cycling reaction, between fructose-6-phosphate and fructose 1,6 diphosphate, which liberates heat with the hydrolysis of ATP. Kammer and Heinrich (1974) have demonstrated that the heat production requirements far exceed the maximum available from such 'futile-cycling'. However it may be that substrate cycling does occur in conjunction with shivering to maintain a high T_{th} (Surholt *et al.* 1990) although this has also been disputed (Heinrich 1993).

Insects demonstrating a preactivity warm-up are best described as facultative endotherms or heterotherms; while normally ectothermic, they can however switch on an endothermic heat generation system in part of their body when it is necessary to become active at a low T_a .

Heat generated during flight muscle activity cannot be controlled (Kammer 1981), although some moths and dragonflies can regulate the heat produced by alternating between gliding and powered flight (Corbet 1963). Therefore any thermoregulation exhibited by an active insect must be due to regulation of heat loss over the body surface. The main avenue for heat loss is convection, the rate of which will vary with size, shape, pubescence and air velocity.

In small uninsulated insects heat loss will be high and so overheating will not be a problem, however for larger insects heat build-up in the thorax may be rapid and can lead to thermal stress. Several different adaptations have evolved that have overcome this. Enzyme systems which can tolerate these higher temperatures have evolved in a few bee species (Heinrich 1980b). Another solution can be seen in some bees with high wing loadings, where heat production is relatively independent of flight speed (Ellington *et al.* 1990). When T_{th} approaches the upper critical limit, cooling can be achieved by increased convection through a higher flight speed (Heinrich & Buchmann 1986). Finally there are physiological mechanisms which are used to facilitate heat loss.

Sphinx moths transfer excess heat from the thorax to the abdomen which, due to the larger surface area and reduced insulation, loses heat rapidly through convection. Heat is carried through the body by the haemolymph and heat loss may be increased or decreased by controlling blood flow between the thorax and abdomen (Heinrich 1971). Bumblebees employ a similar mechanism using a counter current exchange system within the petiole that retains heat in the thorax.

This counter current mechanism may be bypassed when T_{th} is high, leading to convective cooling from the abdominal thermal window (Heinrich 1976). Honeybees also possess a counter current heat exchanger in the petiole, the anatomy of which differs from the bumblebee, and overall seems to play a less important role in heat dissipation. However honeybees can also regurgitate fluid from the honey-crop which cools the head by evaporation and so sets up a heat gradient which helps regulate T_{th} (Heinrich 1980a).

May (1976a) and Bartholomew (1981) both predicted that body mass would be an important determinant of warm-up rates and T_{th} . Within the Apoidea it has been shown that there is a strong positive correlation between body mass and these two factors, once the effects of thermal regime and phylogeny have been removed (Stone & Willmer 1989b). This can largely be explained by the change in the surface area to volume relationship through the range of masses. Warm-up rates depend upon the balance between rates of heat gain and heat loss, both of which are differentially affected by changing body mass.

For a given bee species, power output per unit mass of thoracic muscle is constant and the proportion of body mass accounted for by thoracic musculature is also constant, so it follows that power output is proportional to body mass (Stone & Willmer 1989b). However, this relationship does not hold true for cross species comparisons, as the thermal environment to which the enzyme systems of the flight machinery are adapted varies considerably. A bee adapted to a relatively warm thermal regime will have a lower warm-up rate at a low T_a than a bee adapted to a cooler thermal regime.

The major avenue of heat loss is through convection. Thus for a given morphotype, the surface area to volume ratio increases as mass decreases and so convective cooling is greater the smaller an insect is (Bartholomew & Epting 1975).

Again this holds true for intraspecific comparisons, but not for interspecific ones where differing levels of insulation and the existence of physiological mechanisms for controlling cooling greatly alter rates of convection between species of similar mass.

The balance between the rates of thermogenesis and convection thus determine the relationship between body mass and warm-up rate. Therefore size strongly influences whether thermoregulation is possible or not. For very small insects convective heat loss is so great that they are incapable of endothermic regulation, even with significant heat production from thoracic musculature (Stone & Willmer 1989b). It has been suggested that bees below 30-40 mg will have restricted endothermic abilities (Stone 1989) because of the excessive cost of endothermic warm-up due to the rapidity of cooling.

If heat production and loss are passive processes then the difference between T_{th} and T_a will remain constant; active thermoregulation however requires the maintenance of a relatively small range T_{th} s over a range of T_a s. Within the insect group, thermoregulatory ability represents a continuum of various levels of behavioural through to physiological mechanisms for T_b regulation. Even highly evolved facultative endotherms use behavioural strategies whenever possible to control T_b , as these are generally the least costly in terms of metabolic energy required.

Being able to thermoregulate gives an insect greater independence from ambient thermal conditions and so can confer several advantages. An individual may remain active at lower ambient temperatures and so expand its habitat range, both in space and time, thus improving foraging (Watt 1968; Chappell 1982) and reproductive opportunities (May 1977; Chappell 1984; Stone *et al.* 1995). Maintaining an elevated T_b increases the rates of certain metabolic processes and

so can enable an insect to: escape from a predator more quickly (Fletcher 1978); catch prey more easily (May 1976b); accelerate rates of egg and larval development (Jeanne & Morgan 1992); and resist viral infections (Tanada 1967).

Heterothermy (facultative endothermy) has been demonstrated in at least 8 insect orders: Odonata, Orthoptera, Neuroptera, Hemiptera, Lepidoptera, Diptera, Coleoptera and Hymenoptera. In most cases an elevated T_{th} is associated with flight; however other activities are associated with facultative endothermy, such as brood incubation in bumblebees (Heinrich 1974), ball rolling in some dung beetles (Bartholomew & Heinrich 1978) and also singing in katydids (Heath & Josephson 1970). Within the Hymenoptera, studies have tended to focus upon the Apidae and particularly the genus *Apis* (Heinrich 1979; Cooper *et al.* 1985; Coelho 1991) and the genus *Bombus* (Heinrich 1972, 1976; Prys-Jones 1986; Surholt *et al.* 1990). Most solitary bees studied have tended to be larger species within the Anthophoridae, such as the carpenter bees of the genus *Xylocopa* (Chappell 1982; Nicholson & Louw 1982; Willmer 1988) and also the genus *Anthophora* (Stone 1989, 1993a). Endothermy has also been found within the larger members of the Andrenidae, Colletidae, Halictidae and Megachilidae families in the U.K. (Stone & Willmer 1989b). Ghazoul & Willmer (1994) demonstrated endothermic warm-up in two species of *Bembix* wasp (Sphecidae).

1.2 A brief review of nest-site selection in Hymenoptera.

Within their biogeographical range, individuals of a given species do not normally nest at random. Instead they choose specific habitats to initiate nesting behaviour. However, the degree of specificity does vary between species, with some using only narrowly defined environmental characteristics and others tolerating a broad spectrum of conditions (Malyshev 1935; Stephen *et al.* 1969; Roubik 1989).

Aggregations of ground nesting Hymenoptera are common (e.g. Evans 1966; Michener 1974). Gregarious behaviour may be the result of natal nest-site fidelity, limited suitable substrate availability, or some other social factors. The importance of both the proximal and ultimate causes for aggregating behaviour will be discussed later in greater detail.

A female invests in the future fitness of her offspring directly by attempting to select the most favourable microhabitat for their development and growth. In addition she must seek conditions that will best allow her to forage, mate and excavate as effectively as possible. For instance, a single parameter such as ground temperature will influence both larval developmental rates, and also the body temperature of the adult female when in the burrow; this in turn will determine how quickly she can warm-up and therefore whether she is able to fly or not.

Nest-site selection must therefore involve a perceptual response to certain cues within a portion of a heterogeneous environment. Precisely which edaphological, meteorological and biological factors involved in nest-site selection have only been looked at for a few species of hymenoptera.

In a study of several species of bank-nesting bees in Brazil (Michener *et al.* 1958), several microclimatic and edaphic factors were measured. Only exposure to the sun and position within the soil profile seemed to be of general importance. It was concluded that female philopatry was probably the primary cause of nest aggregations, as adjacent sites of similar characteristics within the embankment remained unused.

The alkali bee *Nomia melanderi* has been shown to select sites possessing certain substrate attributes in order to establish successful colonies (Stephen & Evans

1960). Aggregations of many thousands of individuals were characterised by soils with less than 8% clay content and a slow surface seepage. A high level of surface salinity was recorded, and this was associated with increased water retention by the soil due to the absence of plant growth in the nest-site. Although many proximal factors intercorrelate well, the ultimate factor is most likely to be the soils ability to maintain high moisture levels thus presenting the most favourable conditions for the brood cells. If the substrate is acceptable then emergent females tended to initiate new nests in close proximity to their natal nest (Stephen *et al.* 1969).

In Japan, *Halictus duplex* (Halictidae) tends to form large aggregations in a limited area provided it is free of vegetation or other surface cover. Microclimatic factors (insolation and humidity) markedly effect the distribution of nests but minute pedological factors (amount of humus, pH, constitution of soil particles) play no important role (Sakagami & Hayashida 1961). The patchiness of the aggregations, within an apparently uniform site, lead to the suggestion that females were exhibiting some sort of natal nest-site fidelity.

Osgood (1972) studied the nesting preferences of several species of *Colletes*, *Andrena* and halictine bees associated with low-bush blueberry in Maine. It was demonstrated that low organic contents (and narrow surface organic horizons) and high bulk densities of the soil were the important physical parameters favouring nesting. The easier the organic layer was to penetrate by digging, the more likely bees were to nest there.

A study of bumblebee preferences for the positioning of artificial domiciles (above ground, on the surface or underground) in Southern Alberta was undertaken by Richards (1978). Some species were found to be specialists in terms of nest-site

selection while others were generalists. The intensity of usurpation and social parasite pressure were believed to influence the choice of site.

Rubink (1978) demonstrated that, for the ground nesting sphecids wasps *Bembix pruinosa* and *Microbembex hirsuta*, the scarcity of appropriate nesting sites resulted in dense aggregations. Of the wide range of factors examined, it was found that the most important physical determinant of nest-site suitability was the temperature of the soil surface. Soil textural parameters and amounts of vegetation cover were also of some significance; however all these characteristics combined did not fully explain the distribution of nests, and it was suggested that some other social factors might be involved.

Detailed study of the solitary hypogeous wasp *Sphex ichneumoneus* (Sphecidae) revealed relatively broad nesting requirements (Brockmann 1979). Aggregations were usually in flat areas of sandy loam soils which were free of vegetation and received at least 5 hours of direct insolation per day. In addition, nest-site fidelity was observed and presumed to be explained by females learning the location of their emergence.

The nesting habitat preference of ten species of Afrotropical *Ammophila* (Sphecidae) were found to be divided into two broad categories. Some species selected densely vegetated 'closed' habitats while others selected 'open' habitats comprising of fully insulated bare ground (Weaving 1989). Each category had its own particular set of substrate and microclimatic conditions associated with it.

Cane (1991) examined the nesting soils of 32 species of fossorial bees from a diverse range of habitats in North America. He found that no bee nested in clay or silt soils and a preference for substrates containing more than a third sand was shown. All the parameters measured (soil moisture, cell depth, female size, soil

particle composition, mean annual temperatures and precipitation) generally varied independently of one another and could not be used to predict which taxonomic group a particular bee came from. Although the various bee taxa possessed Dufour's gland secretions with markedly differing chemistries, they all performed the same basic function, namely that of nest cell moisture homeostasis. Therefore the type of secretion did not necessarily constrain a particular group of bees to a given range of edaphic and microclimatic conditions.

Jeanne & Morgan (1992) provided a selection of artificial nesting boxes for a temperature zone *Polistes* wasp to nest colonise. It was shown that there was a preference for initiating nests in warmer sites which then produced broods with shorter development times that emerged earlier in the season. The resulting fitness advantage of this was higher expectancy of survival of the colony and greater overall productivity.

In many of the studies summarised above, the factors associated with favourability of a particular site are often related secondarily to other edaphic or microclimatic variables rather than being important in themselves. A searching female may not therefore need to perceive the ultimate factor determining nest-site suitability, but simply use one or more proximate cues that are closely correlated with it.

The establishment and expansion of an aggregation of hypogeous hymenoptera may result from one of several mechanisms. All the mechanisms produce similar groupings of nests (although the relative spacing within the aggregation may differ), however it is difficult to determine which is the underlying cause. In some cases a combination may be responsible; especially if the original behaviour bringing nests together then allows selection to act on other social behaviours so as to reinforce the aggregating tendencies.

The first possibility is that offspring are exhibiting philopatric behaviour. Individuals often return to nest in the vicinity from where they emerged, such that an aggregation develops near a successful nest of the previous year (Michener *et al.* 1958, Sakagami & Hayashida 1961; Sakagami and Michener 1962; Brockmann 1979; Yanega 1990). Malyshev (1935) states that the tendency for bees to re-nest in close proximity to their parent's nest is one of the main causes of gregariousness. This behaviour has a selective value since a nest-site successful enough to produce adults in former years is likely to be suitable again in following years and thus avoids the risk of failing to locate another suitable nest-site. The actual characteristics used by the individual to recognise and return to the natal nest-site are not well known although visual landmarks and chemical odours may be the proximal cues involved (Michener 1974).

A second mechanism relies upon a female 'learning' the general characteristics of the habitat from which she emerged and then nesting in similar conditions (Stephen *et al.* 1969). In this way an aggregation will be established in an area of similar attributes, which will very often be adjacent the natal nest-site itself. This differs from philopatry in that it relies on more general rather than specific environmental cues. For instance, *Megachile rotundata* adults tend to select nesting holes similar in texture and form to those from which they emerged (Tigari 1963). This type of habitat selection will be more marked if the area of available substrate is limited. Nests thus will become crowded together due to the common attraction of local environmental conditions. However limitations in suitable nest-sites often appear inadequate to explain aggregations in many hymenopteran systems (Batra 1978, Eickwort 1981).

Finally, various social factors may be responsible for the formation and growth of aggregations. A large number of nests in close proximity to each other may

present a large number of opportunities for predators and parasites alike. Many parasites that attack aggregations of ground nesting hymenoptera result in directly density dependent (DDD) mortality; such that the areas of highest nest density experience the highest levels of parasitism (e.g. Lin 1964; Lin & Michener 1972; Eickwort 1973; Brockmann 1984; Rosenheim 1987). When parasitic attack is DDD, it would be expected that any tendency to nest gregariously would be selected against unless the overall mortality due to parasitism is very low relative to other factors associated with, and favouring aggregated nesting.

In terms of parasitic mortality, there may be certain advantages to an individual nest in being part of an aggregation. Even though the probability of discovery may be greater for a group of nests when compared to an individual nest, there could be a dilution effect operating. The probability of an individual nest being destroyed or its contents being parasitised is greatly reduced by being part of an aggregation. This process is analogous to predator satiation; since parasites may be constrained by the availability of mature oocytes or handling time to parasitise each nest successfully (Rosenheim 1990). This is a form of inversely density dependent (IDD) mortality where nests in areas of the highest nest density experience lower probabilities of being parasitised with respect to nests in areas of lower density (e.g. Alcock 1974; Rubink 1978; Freeman 1981; Wcislo 1984; Willmer 1985, Evans *et al.* 1986). Endo (1980) suggests that the spider wasp *Episyron arrogans* (Pompilidae) nests in high densities and synchronises nesting activity as such a form of anti-parasite adaptation. The encounter-dilution effect of animal grouping for protection from parasitic flies is reviewed by Mooring & Hart (1992).

Finally, mortality may be density independent (DI) where the levels of parasitism remain unchanged across a range of nest densities (e.g. Field 1987; Rosenheim 1987). The three types of mortality (DDD, IDD and DI) can be identified in the field by comparing the levels of parasitism sustained in areas of differing nest

density. DDD predicts that mortality will increase with density; IDD predicts that it will decrease with density and DI predicts that it will be independent of density.

Gregarious behaviour may be the result of a form of cover seeking or selfish herding as proposed by Hamilton (1971). Each individual female attempts to minimise her 'domain of danger' by attempting to nest as centrally as possible within an aggregation. Central locations should be the safest, as other nests will act as buffers to incoming predators or parasites and so are more likely to be destroyed or parasitised. There is much evidence of selfish herding in the positioning of bird nests (e.g. Tinbergen 1951; Lack 1968), the schooling of fish (e.g. Gross & Macmillan 1980), mammals (e.g. Darling 1937), caterpillars (Tinbergen 1953) and aphids (Hamilton 1978). However there are no studies showing this effect occurring within groupings of insect nests. It has never been shown that peripheral nests are more vulnerable than central ones, although Wcislo (1984) and Larsson (1986) interpreted nesting aggregations as selfish herds. Predatory ants are important natural enemies of ground nesting hymenoptera, Rosenheim (1990) suggests that in this case, central nests may be less prone to attack.

Since the presence of a concentration of nests is more than likely to attract a large number of parasites, it can then be argued that this might favour some sort of group defence to evolve (Lin & Michener 1972). Several host bees have been observed attacking a single parasite within a nest (Thorp 1969) and neighbouring nests have been defended by individual bees (Batra 1978). In halictine bees establishment of colonies has almost certainly been encouraged by parasite and predator pressure (Michener 1958; Lin 1964). This has however been largely through the evolution of sociality (leading to multiple occupancy) within nests rather than co-operative defence between neighbouring nests. Rubink (1978) states that high densities of wasp nests and active individuals may be

advantageous in providing “physical protection from parasites”; he does not however explain exactly what is meant by this. Many parasitic diptera may however be disturbed, from waiting at a nest entrance for an opportunity to oviposit, by bees flying to and from neighbouring nests (pers. obs.).

Predator confusion may be a benefit that grouping produces. Prey capture by vision based predators tends to be more difficult when prey forms tight congregations, as predators find it difficult to focus on a single individual. Many parasites visually track hymenoptera returning to their nests (Batra 1965, McCorquodale 1986, Spofford *et al.* 1986), and this has led to the evolution of complex evasive manoeuvring displayed by many wasps and bees upon approach to the nest. These flights might also be interspersed by periods of remaining motionless, where a visual tracking parasite will have its attention diverted to other individuals (Alcock 1974, Evans & O'Neill 1988).

Two other possibilities have been suggested for the formation and maintenance of an aggregation. Michener *et al.* (1958) thought that the ease of finding mates might be important, such that newly emerged females could mate and start nesting activities without being delayed in searching for a mate. Stephen *et al.* (1965) observed that many solitary and social bees nest in close proximity to food sources, and so tend to aggregate even though other large areas of suitable ground may remain unused. Neither of these suggestions have however been properly substantiated by further studies, and both would appear to be simply a coincidental ‘bonus’ of being aggregated and not the actual factor responsible for the aggregation in the first place.

All the above listed factors selecting for gregarious nesting (philopatry, habitat preference, predator/natural enemies) are all ultimate causes and not mutually exclusive. They are usually very difficult to disentangle from one another.

However, the proximal cues used by individuals, when they decide to join an aggregation, are much more easily established. Many authors have documented the importance of both visual and odour cues.

The presence of other individuals or their nests may provide a visual stimulus for further nesting in a given locality (Rubink 1978). Michener (1974) noted that during the nesting season of a *Nomia* spp., a patch of ground was occasionally investigated but not selected by individuals for a period of days, then within 24 hours of the first tumuli appearing over 10,000 more nests were initiated. This resulted in many nests being dug in adjacent areas of unfavourably dry soil, even though much more suitable soil was available in the close surroundings. Subsequently many nests were abandoned, even after considerable investment. This demonstrates that gregariousness can sometimes outweigh a preference for selecting the most suitable substrate.

This kind of social facilitation may often have an adaptive significance. If an animal is constrained by time then natural selection should act to reduce any prolonged searching behaviour for nest-site location (Brockmann 1979). This may well be true for many hymenoptera which have short nesting periods; for instance any individual that can devote more time to digging and foraging, by letting other individuals carry out lengthy searching behaviours, will be at a reproductive advantage. As we have seen however this strategy may backfire for some females, as in the case of the *Nomia* spp. described above.

Pheromones promoting the aggregation of nests have been proposed. *Amegilla salteri* (Anthophoridae) females are attracted to earth taken from a nesting aggregation of this species, even when it is covered by a cloth (Michener 1960; Cardale 1968). Many European *Andrena* spp. nest in dense aggregations, and if young females are removed and then released at a different point, they will tend

to rejoin the aggregation at the point of release rather than their capture point (Michener 1974). Although this evidence is fairly circumstantial, it does indicate that it is not the memory of the birth place that is important here but some other factor such as odour. Cane (1983a) has shown that pheromones from the Dufour's gland might be involved in gregarious behaviour, as well as aiding females recognise brood cells and other nest individuals.

Other olfactory properties of Dufour's gland secretions include attracting males, aiding some kleptoparasites in locating host nests (Cane 1983b) and most importantly providing satisfactory moisture homeostasis across a broad range of edaphic and climatic conditions (Cane 1991).

The relative spatial distribution of nests within an aggregation may often indicate the presence of interactions between the nesting female and her conspecifics or parasites. If no interaction is occurring then a purely random distribution of nests in space would be expected. An over-dispersed pattern is demonstrated by individuals attempting to maximise the spacing between adjacent nests in a limited area. This has been observed for two different species of ground nesting wasps (Rubink 1978; Brockmann 1979) and was due to interspecific competition. Most hypogeous insects nesting in aggregations will try and space themselves out in order to prevent neighbouring nests from collapsing into each other. Clumping of nests might result from selfish herding tendencies, or from the need to conserve materials as demonstrated by some mason bees *Chalicodoma siculum* (Hefetz, 1992). It is extremely important to distinguish between density and spacing. For instance philopatry, selfish herding and limitations in suitable nesting substrate can all produce aggregations (clumping) within a habitat; however the actual spacing between nests may be regular due to some other factor, such as conspecific aggression.

1.3 Some general aspects of the Halictidae

This is a very large family comprising many small and medium sized bees distributed throughout the Old and New World. Members of this family are recognisable by the reduced mentum and submentum of the labium (Michener 1944). The vast majority of species excavate nests in the ground, but a few North American species use rotting wood as a substrate. Aggregations are common as many species nest gregariously; indeed the name *Halictus* was appropriately derived from the Greek ἀλίζω, meaning 'to crowd or collect together'. In the Tropics halictids are commonly referred to as 'sweat bees', owing to the fact that they are often drawn to human perspiration in hot weather; this behaviour is however, not normally observed in temperate regions.

The Halictidae are divided into three sub-families: Dufoureae, Nomiinae and Halictinae. Identification of species can be extremely difficult and it is sometimes necessary to refer to characteristics of nest architecture in addition to the usual anatomical characters. The Dufoureae are a small group found in Eurasia, Africa and North America; it is still debatable, however, whether this sub-family actually belongs within the Halictidae at all (O'Toole & Raw 1991). The Nomiinae are a large sub-family distributed throughout the Old World Tropics, the Mediterranean and North America. The majority of species are solitary, although a few are parasocial (colonies with adult bees of a single generation). The Halictinae are found across the world and sociality has evolved repeatedly within this group (Wilson 1976). A large number of species are solitary, a few are communal and many (more than a thousand) are primitively eusocial. The most important genera of this sub-family are *Halictus* and *Lasioglossum*; in addition there are also two genera of kleptoparasitic or cuckoo bees (*Sphecodes* and *Paralictus*).

From the above overview of the sub-families, it is apparent that halictids exhibit a continuous spectrum of social organisation (Table 1.1). Within a single genus, *Lasioglossum*, a full range of social behaviour is displayed (from solitary all the way through to primitively eusocial). The general classification of social organisation may be somewhat confusing as the broad categories used are not always discrete, and this is especially true when considering eusociality. Attempts to resolve these problems by examining eusociality as a continuum have been made recently (Sherman *et al.* 1995; Crespi & Yanega 1995); but for the rest of this study the terminology used by Michener (1974) is kept to, as comparisons with other works are made easier. Even a single species can show great variation; for instance some populations of *H. rubicundus* can show a mix of primitively eusocial and solitary lifestyles (Yanega 1988) and others a purely solitary existence (this study). Michener (1974) comprehensively reviews some of the early and massive literature on sociality in various halictid species. More recent accounts include descriptions of solitary nesters (e.g. *Halictus tsingtouensis*, Sakagami 1980; *Augochlorella striata*, Packer *et al.* 1989b; *Lasioglossum figueresi*, Wcislo *et al.* 1993); communal nesters (e.g. *Agopostemon virescens*, Abrams & Eickwort 1980) and primitively eusocial nesters (*Lasioglossum laticeps*, Packer 1983; *Halictus ligatus*, Packer & Knerer 1986; *Lasioglossum cooleyi*, Packer & Owen 1989a). In addition several species show geographic variation in the degree of sociality expressed (*Halictus ligatus*, Michener & Bennett 1977; *Augochlorella striata*, Packer 1990; *Lasioglossum aeneiventre*, Wcislo *et al.* 1993).

Across the Halictidae, the majority of temperate species have nests that are founded singly (haplometrosis) by inseminated females that have emerged from their overwintering burrows (Sakagami & Hayashida 1958; Packer & Knerer 1986; Wcislo *et al.* 1993). A period of nest construction and provisioning then follows which results in the production of the first brood. For some species, typically in cooler climes, this may be the only brood and will contain both males and females.

Many halictid species are continually brooded and multivoltine, especially when the reproductive season is not temporally constrained by the prevailing climatic conditions (Michener 1966; Packer 1986a; Packer & Knerer 1986). There is often more than one brood per year with some species having up to four (for example *L. zephyrum* may have three or even four broods per year in the US (described in detail by Batra 1964, 1966). For the more social species the first brood will be predominantly if not all female and will constitute a worker caste. As with other social species of bee, the fact that there are no males to mate with in the first brood thus 'predisposes' the females to remain within the nest as non-reproductives. Effectively the nest foundress has manipulated her daughters into assisting her in the production of subsequent broods.

Workers assist in extending the nest and provisioning the new cells; the existing queen may aid in this process or simply remain in the nest only to oviposit. In some species the workers can be fertilised and may contribute genetically to the subsequent generation(s); they may even become replacement queens if the foundress is lost. Nests can contain, depending upon the species, from two to several hundred individuals, which occupy a single burrow. In most of the eusocial species the queens and workers can only be distinguished from each other by more physiological traits, such as ovarian development, and by behaviour (Wilson 1986). The mating behaviour of male halictine bees is also highly variable and is reviewed by Barrows (1976a, b).

The above general outline of halictid life history is very broad and does not do justice to the subtle and intricate variations that exist in the multitude of species that have been studied. This is, however, beyond the scope of this thesis. The life history of *H. rubicundus* is described in chapter 3 with references to other works.

1.4 Aims of this thesis

A wide variety of halictid bees have been studied in the US; in contrast comparatively little attention has been paid to British species (except *Lasioglossum laticeps* by Packer (1983)), and especially the three native species of *Halictus*. As *H. rubicundus* is found throughout the UK and is a common visitor to many flowering plants, it provides an ideal bee for study. As little is known about this bee's general biology, the first aim of this thesis was to investigate this in detail and the findings presented are in Chapter 3. Particular attention is paid to the nest architecture, size variation across UK sites and the (klepto)parasites associated with this species. The influence of the parasite presence is examined with respect to foraging behaviour (Chapter 3) and nest-site selection behaviour (Chapter 5).

The second objective of this study is to examine the thermal biology of this species and ascertain which behavioural and/or physiological strategies are important in thermoregulation (Chapter 4). At this time there does not appear to be any studies specifically investigating the thermal biology of any halictid bee. Stone & Willmer (1989) do however include three species from Halictidae in their broad study of endothermy in the Apoidea. In this thesis an initial examination of the biophysical determinants of heat exchange is carried out (section 4.1), with a focus upon the influence of size and gender (section 4.2). The variability in the microhabitats available to *H. rubicundus* at the nest-site is considered (section 4.3), and the influence of microclimate on flight activity discussed (section 4.4). Together these investigations are combined to explain a variety of behavioural patterns displayed by each sex (section 4.5).

Several studies on bees have investigated a small selection of possible factors influencing nest-site selection. The aim of Chapter 5 is to quantify the relative

importance of a comprehensive array of biotic and abiotic factors that determine the suitability of a nest-site. Variations within a single site and across a number of sites are considered and the key elements for each highlighted. The two-dimensional patterns of nest spacing within an aggregation are similarly investigated and the determining factors discussed.

Chapter 6 brings together several aspects of the behavioural ecology of *H. rubicundus* examined in Chapters 3, 4 and 5 (sections 6.1 to 6.3). Discussion of the potential use of this kind of study for improving crop pollination is given in section 6.4. The encouraging of native bee species, such as *H. rubicundus*, to nest near plantations, and the introduction of novel crop species to an area are further evaluated. The sociality of *H. rubicundus* and other halictid species is described in section 6.5, and the importance of various microclimatic and ecological influences on social structure are discussed.

Table 1.1. Levels of social organisation among bees (based on Michener 1974). '+' indicates that column heading applies, '-' indicates that it does not, '±' indicates variability. * gynes, if any, can survive alone.

Level	Castes and division of labour	Colonies with adults of two generations	Cooperative work on cells	Females structurally similar *	Progressive feeding	Frequency of occurrence within the Halictidae	Example from the Halictidae
Solitary	-	no colonies	-	+	-	very common	<i>H. quadricinctus</i>
Subsocial	-	-	-	+	+	never	-
Parasocial							
Communal	-	-	-	+	-	occasional	<i>N. punctulata</i>
Quasisocial	-	±	+	+	±	very rare	<i>N. capitata</i>
Semisocial	+	-	+	+	±	rare	<i>L. versatum</i>
Eusocial							
Primitively	+	+	+	+	±	common	<i>L. zephyrum</i>
Highly	+	+	+	-	±	never	-

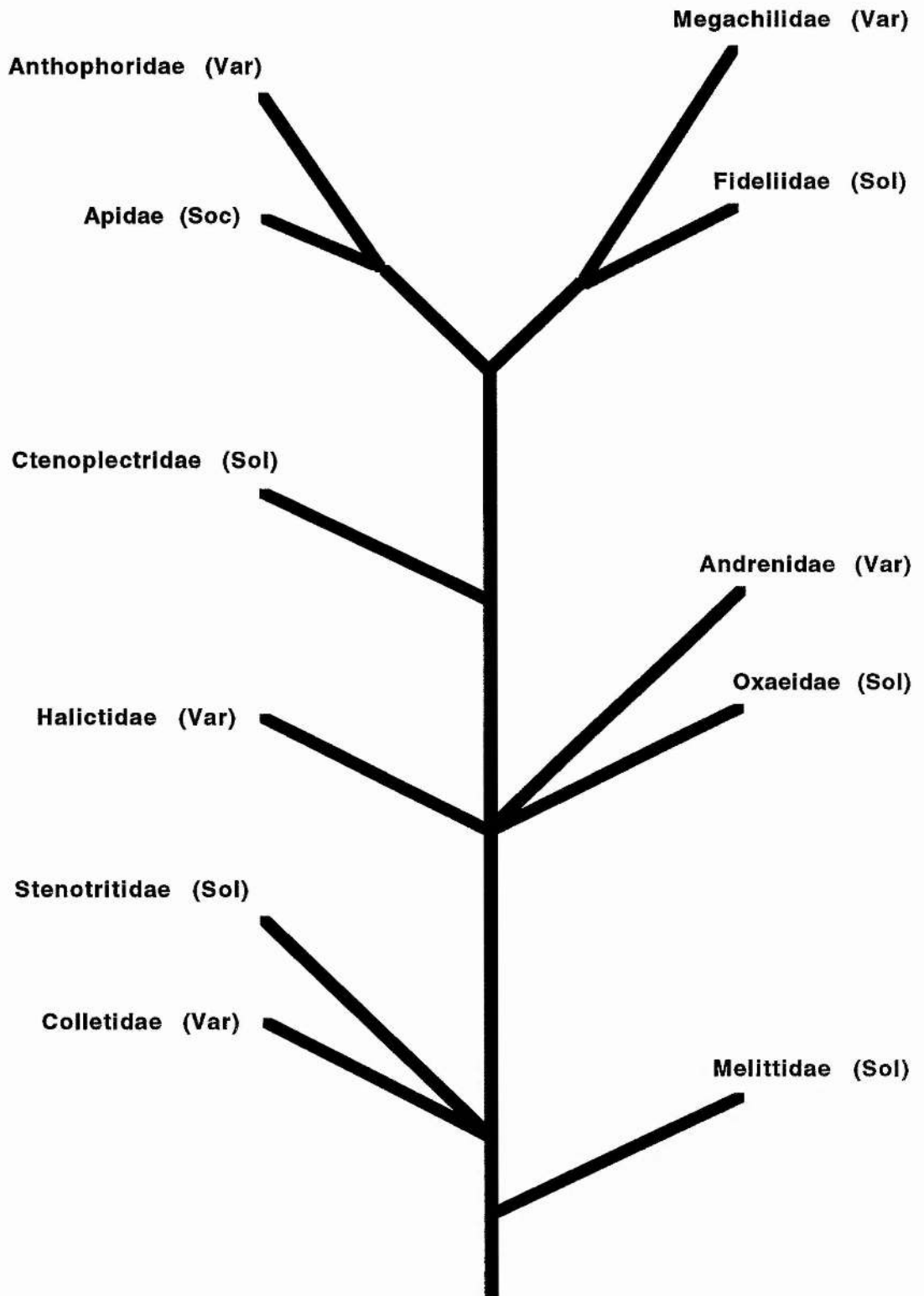


Figure 1.1. A simple evolutionary relationship of families within the Apoidea. Sol = families with only solitary species; Var = families where the majority of species are solitary but also containing species with higher levels of social organisation; Soc = family containing mainly eusocial species (adapted from O'Toole & Raw 1991).



Fig. 1.2. Female *H. rubicundus* on ground near nest entrance (from O'Toole 1991).

Chapter 2 Sites, materials and methods

2.1 Field sites

Although *H. rubicundus* is a relatively abundant bee throughout the UK, it is difficult to locate aggregations of this species. After intensive searching of over 80 sites, possessing suitable habitat characteristics for ground nesting Hymenoptera, 10 nesting aggregations were discovered (Figure 2.1). At several other sites *H. rubicundus* was observed flying or foraging, but no nest-site was found. Some general information about the 10 study sites, in 1994, is summarised in Table 2.1. The following abbreviations are used for each site in later figures:

I	Invergowrie
Te	Tentsmuir East
Tw	Tentsmuir West
C	Chatton
N	Newcastle
K	Kildale
G	Gibraltar Point
S	Swindon
Pn	Prinsted North
Ps	Prinsted South.

2.1.1 Invergowrie

This was the primary field site used in this study and the place where the majority of behavioural investigations were carried out. It is situated in the grounds of the Scottish Crop Research Institute (S.C.R.I.) at Invergowrie (Figure 2.2a). There were several hundred *H. rubicundus* nests in a gently sloping banking which was 3

m wide by ≈ 80 m long. This species of bee has been known to nest there for "several years" according to the farm manager at the S.C.R.I.. The banking was rebuilt in 1976 to support the elevated road that runs along the top of it; and presumably the present aggregation has built up since then.

The banking was at an approximate angle of 30° to horizontal and had a southern aspect; to the north it was delimited by the road and to the south by grass. The details of the substrate and other features of the site are described in chapter 5. Figure 2.3 shows the banking in relation to the various crop plants situated on the S.C.R.I.. To the south there was an experimental plot of raspberry (*Rubus idaeus*) and to the north a field of barley.

The eastern part of the banking contained the highest density of nests and was used for the majority of studies. An area of 3 m by 42 m was divided into 1 m^2 quadrats using fine fishing line to aid mapping of the nests and to delimit set areas for behavioural observations. The density of nests in 1993 is given in Figure 2.4. During 1992 a single andrenid nest was situated in quadrat 41B and an ants nest persisted in quadrat 11A for the length of this study.

2.1.2 Tentsmuir

On the south eastern side of Tentsmuir forest there are a series of fields used for cattle grazing. Rabbits have extensively burrowed into the sides of grassy mounds causing massive erosion and the production of a dune system (Figure 2.2b). Much of the exposed soil is in the form of vertical sandy bankings and *H. rubicundus* had colonised two south westerly facing cliffs (approximately 300 m apart). The eastern aggregation covered an area of 0.8×12 m and the western aggregation an area of 0.7×8.5 m. This was a highly disturbed site, as the dunes are eroded back by up to 0.5 m per year (pers. obs.), and there were often large

areas of collapsed banking (exposing nest cells) after heavy rains. Other hymenopteran species using these dunes included two *Andrena* sp. and the sphecoid wasp *Mellinus arvensis*. In addition to the grass there were many gorse bushes (*Ulex* sp.) and also thistles (*Cirsium* sp.) present.

2.1.3 Chatton

A section of embankment, on the east side of the River Till in Chatton, supported an aggregation of *H. rubicundus*. It was found in a 1 m high vertical sandy bank with a westerly aspect, and the aggregation occupied about 12 m of its length (Figure 2.2c). A few isolated nests were also found in another piece of banking facing south east. There were several large aggregations of *M. arvensis* on other similar sections of the embankment further upstream, and the cuckoo bee *Sphecodes gibbus* was a common visitor to the aggregation (see section 3.8.2). The surrounding vegetation is mainly grass with some gorse (*Ulex* sp.), groundsels and ragworts (*Senecio* spp.).

2.1.4. Newcastle

The River Tyne is bounded by large sand and clay cliffs along a section west of Newcastle. The area lies within Close House Riverside S.S.S.I. and contains a wide range of flowering plant species. The near vertical cliffs face south and so capture the majority of the available sun; this makes them suitable habitats for aculeates. Many species of bees and wasps can be found nesting in the cliffs including several andrenids, halictids and a colletid. Two species of *Sphecodes* were often observed investigating burrows in the cliffs. The aggregation of *H. rubicundus* was somewhat dispersed across a 120 m section of cliff which varied between 1 and 3 m in height (Figure 2.2d). There were several small aggregations interspersed with more isolated nests throughout this area.

2.1.5 Kildale

Kildale is an area of moorland situated in the north western part of the North Yorkshire Moors. Several eroded sandy bankings of River Sleddale face due south and supported aggregations of *H. rubicundus*. The largest was a near vertical piece of banking 2 m x 1.8 m (Figure 2.2e). Adjacent to the banking the vegetation was grass and the predominant flowering plant in the area was heather (*Calluna vulgaris*).

2.1.6 Gibraltar Point

Gibraltar Point Nature Reserve covers an area of 430 hectares comprising sand dunes, salt marshes and freshwater habitats. An eroded path running 600m along the West Dunes has more than 500 nests present in small dispersed clumps. The Reserve also has records of at least 23 other species of Apoidea and numerous species of flowering plants (C J Hawke, pers. comm.).

2.1.7 Swindon

Coates Water Country Park contained a small aggregation in a section of exposed banking. The sandy soil was inhabited by 36 female bees and there were in addition several *M. arvensis* nests in the same area. The park as a whole contains a wide variety of flowering plants and is a well known aculeate site (R Gabriel, pers. comm.).

2.1.8 Prinsted

Two aggregations were found in the dune system of Thorney Island. The first was on an eroded pathway with a gently sloping southern aspect (Figure 2.2f). It

covered an area of 5 m x 1.5 m and was surrounded by mixed grassland. The second site was on the south east facing banking of a small stream and covered 2 m x 1.2 m. The site was approximately 20 m south of the first and contained more nests (Table 2.1). A nesting aggregation of *Halictus tumulorum* was also found on the main pathway running to the north of these two sites.

2.2 Marking

2.2.1 Marking bees

In order to carry out detailed behavioural observations it was necessary to mark many bees individually. This was done by catching individuals as they exited a nest using emergence traps (Figure 2.5) or using a hand net. The bee was then placed in a glass vial and a small amount of CO₂ from a portable cylinder (BDH Ltd, UK) was introduced to anaesthetise the individual. With practice, enough CO₂ could be used to anaesthetise the bee just long enough to allow marking, thus causing minimal disturbance.

Once narcotized, the bee was placed in a plunger type queen marking cage (Steele and Brodie, UK) modified with a finer mesh to accommodate smaller bees. Five colours of quick drying queen marking paint (Steele and Brodie, UK) were then used to mark each individual; the 2.5 mm diameter numbered queen marking discs were found to be too large for this application. Using five colours and ten positions (Figure 2.6a) it was possible to mark each individual uniquely (Figure 2.6b). The minimum amount of paint was used and applied with a very fine brush and care taken to avoid spreading paint onto the wings, tegulae and head. A marking system was used that ensured that if a coloured spot was lost, an incorrect identification could not be made; such an individual would be automatically excluded from any analysis that required a positive identification

(Southwood 1978). The paint spots were fairly durable and seventeen marked females were still identifiable after overwintering.

After marking, an individual was allowed to recover in a cool and sheltered area and would fly off after only a few minutes. Other data were often collected at the same time that marking was undertaken. This included mass, head width and pollen samples (see section 2.9).

2.2.2 Marking nest entrances

At Invergowrie, nests were marked with numbered drawing pins and these were placed 10 mm due north of the nest entrance. All nest entrances were marked with a plain drawing pin initially and only when determined to be an active nest was a numbered pin used. A nest was classed as active if a female was seen to return there with at least one pollen load. Other burrows were marked separately if used only for sleeping by males.

2.3 Behavioural observations

The majority of studies were carried out at the Invergowrie site, with occasional additional work being carried out elsewhere. These involved the observation of many marked females for several hours per day for many days throughout the season. All times quoted are for British Summer Time (BST) and duration of activities were recorded in minutes and seconds. The behaviours observed are defined below:

2.3.1 Nest entry and nest exit

Foraging time was the duration between a female exiting a nest and returning to the nest with a visible pollen load. Females returning without pollen may have been carrying nectar from a flower visit; this could also be classed as a foraging trip but was not included in the analysis since it was impossible to tell, with this study, whether a female was carrying nectar or whether she had left the nest for some other reason (for example to mate).

Time in nest was defined as the duration between the entry and subsequent exit of a female. The activities being undertaken by the female within the nest were unknown except for digging, since there were no visible cues on the surface to indicate what was happening underground. When females pushed loose soil out of the nest entrance it was assumed that some sort of excavation was taking place; it was not possible however to observe whether this was digging to extend the nest or to construct a new cell.

Data on foraging time and time in nest were collected on 12 separate days through the main female provisioning phase (May, June and July) of 1993. This comprised 33 separate individuals some of which were continuously observed for whole days, thus allowing nest cycles to be constructed (see section 3.3).

Time of first emergence of a female from the nest was recorded on several days throughout the season for several different females.

Basking time was recorded for males and females remaining motionless on the ground or in the nest entrance before flying off. To be classed as a basker the individual would have to be in direct sunlight and have its body oriented to the direction of incoming radiation. In addition to the basking duration, the type of

substrate and several microclimatic variables (see section 2.5.2) were also recorded.

When a female returned to her nest the presence or absence of a pollen load was noted and also the number of pursuing dipteran parasites was recorded.

2.3.2 Abundance

The number of females, males and parasitic Diptera flying in a 1 m² quadrat was counted using a hand tally (BDH Ltd, UK). This was done for 10 minute periods every half hour for the entire activity period of the day. Measurements of microclimatic conditions were also recorded for the corresponding periods.

2.4 Size measurements

In the field head width was used as an indicator of size since it was easily and quickly measurable and caused the least amount of disturbance to the individual. Width was taken from an anaesthetised bee to the nearest 0.01 mm using a hand held digital micrometer (Mitutoyo Corporation, Japan).

Measurements were checked for specimens returned to the laboratory using a binocular microscope with an eyepiece graticule (Meiji Techno Co. Ltd, Japan). Differences in field and laboratory measurements were small (all less than 0.02 mm) and non-directional.

Mass was also measured in the field (for 40 females and 48 males) to the nearest 0.5 mg using a hand held microbalance (Unwin 1980). Head width and live mass are highly positively correlated for both sexes (males: $p < 0.001$ and females: $p < 0.001$) and this is discussed in section 3.7.1.

2.5 Weather and microclimate measurements

The microclimatic conditions a nesting aggregation experience have important influences on the behaviour of bees (chapters 3 and 5). There are, however, considerable difficulties in comparing microclimate measurements from sites spread across the UK. Since readings would have to be taken on different days, variation in weather conditions would confound any direct comparison of results. It was therefore decided that comparisons of prevailing weather conditions, averaged over several years, would be a much more useful data set for analysis. Microclimatic variables were, however, measured for use with behavioural studies within a site.

2.5.1 Weather data

For the 10 field sites across the UK, data about the prevailing weather conditions were compiled from the monthly journal 'Weather' published by Royal Meteorological Society. The weather stations closest to each of the field sites were used (Table 2.2) and in order to establish what the prevailing weather conditions were, data from 1988-1992 were averaged. At Gibraltar Point the Nature Reserve's own weather station data were used.

Mean maximum and minimum monthly temperatures were calculated for the flight season of *H. rubicundus* (May to September). These were adjusted for altitude differences between the field site and the weather station (Langmuir 1984). In addition the mean monthly hours of sunshine and mean monthly rainfall totals were also calculated for the flight season.

2.5.2 Microclimate data

Several microclimatic variables were measured throughout the activity periods of *H rubicundus*. A four channel data logger (Squirrel meter/logger, Eltek Ltd, UK) was used to record ambient temperature (T_a), ground surface temperature (T_g), nest entrance temperature (T_n) and solar radiation (L). These could be automatically logged at any desired interval (usually every 30 minutes) throughout the recording session or read spontaneously from the display readout whenever required.

Temperature measurements were obtained using standard type U mini-thermistors (Grant Instruments Ltd, UK) located in different positions. T_a was measured 100 mm above the ground surface in the shade; this is the height at which the bees usually flew above the banking (pers. obs.). For T_g the thermistor was laid on the ground surface and for T_n the thermistor placed in the entrance of a burrow. Solar radiation was measured using a silicon cell pyranometer (Skye Instruments Ltd, UK) which was placed on the ground so that the sensor was absolutely horizontal and away from any shading.

Relative humidity (RH) measurements were obtained from a hand held humidity meter HMI 31 (Vaisala Ltd, UK), which was placed 100 mm above the ground surface in a shaded position. Wind speed (W) was obtained from a hand held anemometer (Testovent 4000, Testoterm Ltd, UK) also placed 100 mm above the ground. This device could be programmed to give a mean wind speed for a 10 minute period, thus giving a single useful value rather than a series of highly fluctuating values.

The solution of energy balance equations used to predict body temperatures of insects are generally tedious and require extensive microclimatic data, the

calculation of derived coefficients, and the making of several assumptions. The use of models of animals and standard operative temperatures (T_{so}) can be used to overcome many of these problems. The models are usually dried insects with a thermocouple inserted into the thorax and possess the size, surface area and colour characteristics of a live individual. Since the models produce no heat their body temperature, T_{so} , will be entirely determined by the environment. This technique allows the effects of radiative, convective and conductive heat exchange to be combined into a single useful measurement. Some studies (Pivnick & McNeil 1987; Corbet *et al.* 1993) have used hollow copper spheres painted black instead of dead insects in an attempt to standardise T_{so} . The black globe temperature will be positively related to T_{so} , and may be useful in interspecific comparisons; however the size of the globe must be appropriately chosen to be an analogue of an insect's surface area.

Differences between T_{so} and the body temperature of an equivalent live animal allow estimates to be made of the amount of physiological heat exchange ($T\Delta$) that is occurring (evaporation is assumed to be negligible). This will include any metabolic heat generated (basal or as a product of some active process) and any physiological thermoregulation such as the control of haemolymph flow around the body (Heinrich 1971). An insect's body temperature is then the sum of T_{so} and $T\Delta$. The T_{so} measurements are best equated to a basking insect rather than a flying individual because the forced convection due to wing beating is not a factor that is incorporated into the model.

Using this technique an animal's environment can be effectively mapped by placing the T_{so} model in various positions, so that the range of thermal conditions an animal is likely to experience can be characterised (Chappell 1982). The effectiveness of particular behavioural strategies for thermoregulation, such as

posturing, have been quantified (Polcyn & Chappell 1986) and the relationship between T_{so} and behaviour patterns studied (Corbet *et al* 1993).

T_{so} s were recorded using a Type K (P9005) thermometer with a modified type K thermocouple (Portec Instruments Ltd, UK). The thermocouple was inserted into the centre of the ventral surface of the thorax of a dried female *H. rubicundus* so that the end was located in the centre of the thorax. The same female was used for all recordings of T_{so} and had a head width of 2.70 mm (mean head width for females at Invergowrie = 2.72 ± 0.01 (159) mm). T_{so} was measured at 100 mm above the ground surface for the same reason T_a was. The use of a dried specimen means that the T_{so} s recorded will not be the same as for a live (or freshly killed) bee; however, they can be used as an index of actual T_b s experienced and so are useful when making comparisons.

For all the above measurements the sensors were set up and allowed to equilibrate for at least 10 minutes before any readings were taken. In addition to the above method of measuring microclimatic variables at a given area, all the above variables could be measured elsewhere for use with 'grab and stab' measurements (see section 2.6). These would be taken immediately after catching a bee, and values recorded once the readout had settled down (usually 5 to 10 seconds). This method of recording allowed microclimatic data snapshots to be taken for a particular bee at a particular point in the habitat.

2.6 Grab and stab techniques

The measurement of the thoracic temperature (T_{th}) of insects in the field is a well developed technique (Louw & Nicholson 1983; Cooper *et al.* 1985; Heinrich & Buchmann 1986; Stone & Willmer 1989b; Stone 1993). In the past this has mainly been used for bees much larger (mostly > 100 mg) than *H. rubicundus* (female mass

= 30.2 ± 1.3 (41) mg). For such small individuals the construction of the thermocouple was modified in order to minimise heat loss along the thermocouple wires and also reduce the time necessary for the thermocouple to attain the same temperature as the thorax.

Instead of using 40 gauge copper and constantan wire (0.15 mm diameter) 48 gauge wire was used (0.04 mm) thus reducing heat conductance (Scientific Wire Co. Ltd, UK). Other workers have made thermocouples employing the hypodermic needle as part of the junction; in this study however the copper and constantan were soldered to produce the thermocouple and then threaded inside the hypodermic needle. The junction was then fixed so that it was just exposed at the end of the needle (D05930, Gillette, UK) using a minimal amount of glue (Superglue gel xtra, Loctite Ltd, UK). This was then wired into a Type T plug and connected to a Type T thermometer (P9005, Portec Ltd., UK). This produced a very fine thermocouple junction which responded quickly to temperature change.

In the field an individual was caught and the thermocouple inserted dorsally into the centre of the thorax within six seconds, otherwise the data were disregarded. The bee was left in the net and held against a piece of styrofoam for thermocouple insertion, thus minimising heat loss. This process was always carried out in the shade to prevent warming from direct sun. Some errors associated with 'grab and stab' have been investigated (Stone & Willmer 1989a) and these are discussed in section 4.5.

The types of activity the bee was displaying was noted at the time of capture and these are defined below. **Flying** meant that an individual had been in flight for at least 5 seconds before capture; **just flying** was when a bee had taken off less than 2 seconds previously; **walking** was taken as an individual that had been moving along the ground surface without flying for at least 2 seconds and **basking** was

recorded when an individual had been motionless on the ground for more than 2 seconds. Particular care was taken not to disturb bees into taking off, and for an individual to be 'just flying' take off had to be an apparently voluntary act. T_a and head width were always measured immediately after T_{th} , and sometimes T_g , L , W and RH were also measured.

2.7 Nest-site characteristics

A wide array of factors was measured at the 10 field sites in the area of highest nest density; in addition these same factors were measured within the Invergowrie site for areas of varying nest density. The within-site study at Invergowrie was carried out during early May 1993, and the other sites visited during May and early June 1994. This time of year was decided upon as it corresponds with the period during which female *H. rubicundus* select areas to found nests.

2.7.1 Nest density

For a nesting aggregation the area of highest nest density was judged by eye and a 1 m² quadrat placed. The number of nests were then counted and this quadrat used for the measurement of other parameters. Mean nest density was calculated by measuring the total area of the aggregation with a tape measure and then counting the total number of nests present using a hand tally. Local nest density was defined as the density of nests surrounding a particular nest; and this was determined by placing a 1 m² quadrat on the aggregation with the study nest located at its centre.

At Invergowrie nest density was determined for 42 one m² quadrats across the aggregation (Figure 2.4). There were also four areas selected for different density

levels and these were classed as: high density, low density, zero density 1 and zero density 2. The high density area was in the east part of the aggregation and comprised 5 quadrats (41A, 40B, 41B, 42B and 41C); the low density area was more central and consisted of 5 quadrats (34A, 33B, 34B, 35B, 34C). Zero density 1 was made up of 5 randomly placed quadrats in the area of grass to the south of the banking; and zero density 2 was 5 quadrats in the eastern most section of the banking outside the marked grid.

2.7.2 General properties of the nest-site

Latitude and altitude were determined from 1:50 000 scale Landranger maps of the relevant areas (Ordnance Survey, UK). Vegetation cover was estimated visually as the percentage of the total area within a 1 m² quadrat covered. Stone coverage was measured by counting the number of stones with a diameter greater than 50 mm. Aspect was taken as the direction the majority of the area of highest density faced with magnetic north being assigned 0°. The slope of the banking was determined to the nearest degree using an Abney level (ex British Army artillery). Three repeats were taken and the mean value used in the analysis.

2.7.3 Substrate properties

Soil hardness was measured in three positions around the centre of the quadrat using a hand held penetrometer (ELE International, UK) and a mean value calculated.

Soil temperature was estimated differently for the 'within site' and 'between site' studies. At Invergowrie, temperature and humidity readings were made on a single day in the quickest possible time in order to minimise temporal changes in these variables (start time = 14:15, T_a = 24.5 °C, RH = 39.8 %). The 42 quadrats

were sampled in a random order to remove any systematic errors. The surface temperature of the ground was measured in quick succession with Type K thermometer. Soil temperatures and humidities were taken at 50 mm depth using two hand held thermometer and humidity meters HMI 31 (Vaisala Ltd, UK). A hole was made in the centre of the quadrat and the probes allowed to equilibrate for two minutes before the reading was taken.

At the other sites it was not possible to measure temperature and humidity in quick succession, so readings were taken through a two week dry period at the end of May 1994. Since ground surface temperature changes very much in accordance with local microclimatic conditions it was not considered a useful parameter to look at across sites. Instead soil temperature excess was used and was defined as the difference between the temperature of the soil at 50 mm and ambient temperature (T_a was always between 18 and 20 °C when the measurements were taken). This is really a crude index of the soil's capacity to absorb solar radiation which will be an important influence in determining nest temperature. Soil humidity was taken at 50 mm, as described above, but was only measured if there had been no rain in the previous 24 hours.

The composition of the nesting substrate was analysed by collecting 50 g samples of soil from the centre of the 1 m² quadrats using a soil corer and subjecting them to the following techniques. Samples were weighed and then dried in an incubator at 105 °C for 24 hours and then re-weighed to establish the water content. Using a 2 mm aperture sieve (Endecotts Ltd, UK) it was then possible to separate the gravel fraction from the rest.

The names of the particulate fractions and size classes are in accordance with British Standard System (BSS) classification. Gravel is defined as particles greater

than 2 mm in diameter; sand as 250 μm to 2 mm diameter particles; silt as 63 μm to 250 μm diameter particles and clay as particles smaller than 63 μm .

The remaining non-gravel fraction was placed in a furnace at 780 $^{\circ}\text{C}$ for 4 hours and then allowed to cool down in an anhydrous atmosphere. The subsequent weight loss gave the mass of organic matter present in the soil. Further sieving of this fraction using apertures of 250 and 63 μm gave the sand, silt and clay component fractions. Standard soil analysis techniques usually use a dispersant (sodium metaphosphate or Calgon) to free particles into their individual fractions. This, however, would not provide data that were as biologically relevant as those above since in the nest-site the female bees would encounter particles in a non-dispersed form. There is a significant difference in the two sets of particle size distributions which was revealed when one sample was also fractionated using a Sedigraph (Micrometrics Ltd, UK) and compared with the above results.

Other soil samples from the same areas were used to determine the inorganic salt content and the pH of the soil. 50 g of soil was well mixed with 50 ml of distilled water and left to stand for 15 minutes. The mixture was then filtered and the filtrate tested for conductivity (a measure of salt content) using a Cond μ - Sensor and for pH using a pH μ - Sensor (both from Whatman International Ltd, UK).

Soil colour was quantified in the field by employing standard Munsell soil colour charts (Kollmorgen Instruments Corporation, US). This gave three colour parameters used to describe the soil; these were hue, chroma and value.

The above techniques are fairly standard for classification of soils and are all discussed in much greater detail by Fairbridge *et al.* (1979) and Fitzpatrick (1980).

2.7.4 Nearest neighbour distance measurements

At each site the nearest neighbour distance of every nest within the 1 m² quadrat of highest nest density was measured using a digital micrometer. At Invergowrie an additional 15 quadrats of varying densities were also surveyed. Within the densest nesting area a further three sub-quadrats (0.1 m x 0.1 m) were placed visually so as to incorporate the maximum number of nests. The nearest neighbour distances were then measured in these sub-quadrats.

2.8 Nest excavations

Nests were excavated during the early morning or when the weather prevented bees from leaving so that the nest contained all the resident bees. Fine talcum powder was first blown down the nest entrance, using a small glass pipette, which coated the main burrow and any open cells. This greatly aided tracing nest structures, especially in densely populated areas where adjacent burrows were in very close proximity.

Excavations were carried out using a variety of small spatulas, mounted needles and paint brushes. The nest architecture was sketched and the dimensions of various structures measured with a digital micrometer. The position of any adult bees and the contents of cells were also noted. Nest contents were often taken back to the laboratory for more detailed examination.

Extensive excavations were carried out at Invergowrie and a few nests were also investigated at Tentsmuir, Kildale and Chatton. It was not possible to dig up nests at other sites, either because the area was a Nature Reserve or the land owner would not give permission.

2.9 Pollen samples

At Invergowrie, pollen samples were collected from two sources: from foraging females returning to the nest and from pollen balls in nest cells. Pollen samples were carefully removed from the corbicula and bodies of anaesthetised females using a paint brush with trimmed bristles. The samples were kept in small glass vials for later analysis. By weighing the female before and after pollen removal it was possible to estimate the mass of the pollen load carried.

Pollen balls were carefully removed from completed cells and dried out in the laboratory using silica gel. It was necessary to do this in order to compensate for the nectar added to the pollen mass by the female bee. This allowed the pollen ball mass to be more directly compared to the forage load mass since both were dry (this does not take into account the sugar content of the pollen ball resulting from the addition of nectar).

Samples from both pollen sources were mixed with pollen stain (acid fuchsin gel) and mounted on microscope slides (Sawyer 1981). Care was taken not to contaminate samples with other pollen sources being prepared. In addition flower pollen samples from oilseed rape (*Brassica napus*) and cultivated raspberry (*Rubus idaeus*) were collected from plants close to the Invergowrie site. The slides were examined under an optical microscope (Prior Ltd, UK) and the percentage of *B. napus* pollen estimated in 5 different views. The number and proportion of other morphologically distinct pollen types in each sample were also noted. This value is likely to be an underestimate of the total number as this technique would be unlikely to distinguish between all the pollen species in the samples. This and other more advanced pollen identification procedures are discussed in Moore & Webb (1978).

2.10 Laboratory investigation of warm-up rates

Bees selected for these experiments were caught in the field and kept in cool dark conditions until needed. The time between capture and use in the experimental set-up was minimal and always less than an hour. After weighing, a 48 gauge copper/constantan thermocouple was attached to an individual in one of two ways. In the first method, a bee would be cooled to 10 °C and restrained in a styrofoam padded vice on a cooled steel stage so that a small hole could be made mid-dorsally in the thorax with a brass entomological pin. A thermocouple was then inserted to less than a mm in depth and secured in place using a minimal amount of glue (Copydex, Henkel Ltd, UK). The depth of the hole was kept as shallow as possible and positioned just off the centre of the thorax in order to avoid damaging the aorta. A second method involved gluing the thermocouple to the thoracic surface of a cooled bee without penetrating the cuticle. Care was taken to make sure that the thermocouple was in direct contact with the thoracic surface and not insulated from it by a layer of glue. All the bees were then returned to the fridge and the glue allowed to set.

The bees were released from the clamp and given a small cube of styrofoam to grasp in their tarsi as they were allowed to warm up to room temperature. The thermocouple was connected to a chart recorder (L 6512, Linseis GmbH, Germany) which continuously recorded the thoracic temperature. In addition ambient temperature was monitored throughout. Flight activity was recorded as were any other activities during the experiment. The errors associated with this set-up are assumed to be small and are discussed by Stone & Willmer (1989a). Further details of this experiment are described in section 4.2.4.

2.11 Measurements of passive warming and cooling rates

To assess the passive heat exchange of bees live, freshly killed and dried dead specimens were mounted on a thermocouple as described in the previous section. A chilled bee ($\approx 10^\circ\text{C}$) was placed in a glass tank to avoid the cooling effect of moving air currents, and allowed to equilibrate with ambient conditions. A 60 Watt bench lamp was placed 100 mm above the dorsal surface of the bee and used to warm the individual to the required temperature. This exposure was equivalent to $\approx 900\text{W m}^{-2}$ simulated solar radiation. The lamp was then switched off and the bee allowed to cool to room temperature. This procedure was repeated three times. For live specimens, the subject was then killed (while still attached to the thermocouple) using an ethyl acetate jar and then the warming/cooling procedure immediately repeated. A characteristic warming-cooling curve was generated on the chart recorder and the initial warming and cooling rates calculated from the curve (Figure 2.7). By plotting cooling rate against temperature excess it was possible to estimate the cooling constant (k) for various individuals from the gradient of the regression line (section 4.2.2).

2.12 Surface area and reflectance

The surface area was estimated by modelling a bee as a series of spheres and cylinders and measuring the appropriate dimensions to the nearest 0.01 mm using a digital micrometer. Only the head, thorax and abdomen were considered as these are the principle structures involved in heat exchange. The legs have a high surface area for their mass, but are assumed to be generally of relatively little importance in heat dissipation. They may, however, increase the effective size of the thorax during flight as a result of being tucked up against the lower side of the body. The effect on the functional surface area of the thorax and consequent modification of heat flux is beyond the scope of this study and is assumed to be

small and is therefore ignored. Reflectance was measured on the dorsal surface of the thorax using a reflectometer according to the procedure described by Willmer & Unwin (1981).

2.13 Statistical methods

All data sets were tested for normality before any parametric test was employed. Data found not to be normal was sometimes transformed (usually by logging) and then re-tested for normality. All means stated are given with their standard errors and the sample size in parentheses. All error bars on graphs are standard errors and numbers adjacent to points indicate the sample size. When used on the same graph, solid lines represent the curve fit for females and dashed line the curve fit for males.

Regression was used to analyse the effect of one continuous variable upon another and the r^2 and p values are given in the text along with the degrees of freedom. Spearmann's rank correlations were employed when a non-parametric test of association was required. To test for a difference in means of two populations a two-tailed Students t test was carried out. For more than two populations one- and two-way analysis of variance was used (ANOVA), and the generalised linear model employed when sample sizes were unequal. Tukey's honestly significant difference test (Pagano 1981) was used for *post hoc* testing the difference between selected means. All these tests are detailed in Sokal & Rohlf (1969) unless otherwise stated and were carried out using 'Minitab' version 8.2 on an Apple Mac, except for the second order polynomial curve fitting which was carried out using 'Graphpad Prism' version 1.00 on a PC. G tests for homogeneity and goodness of fit were in accordance with Fowler & Cohen (1990) and computed by hand. All the circular statistics are by the methods given in Zar (1984).

Table 2.1. Summary of the locations of the ten field sites used in this study. The number of nests gives an estimate of the total number of nests comprising the aggregation and is based on a visual survey carried out in May 1994.

<u>Location</u>	<u>Grid Ref.</u>	<u>Latitude</u>	<u>Altitude</u>	<u>No. nests</u>
Invergowrie	NO 337297	56° 27'	19 m	840
Tentsmuir East	NO 474234	56° 24'	9 m	120
Tentsmuir West	NO 472232	56° 24'	9 m	100
Chatton	NU 062284	55° 33'	47 m	500
Newcastle	NZ 138656	54° 59'	8 m	3280
Kildale	NZ 637097	54° 29'	180 m	221
Gibralatar Point	TF 557584	53° 06'	4 m	575
Swindon	SU 178824	51° 32'	110 m	36
Prinsted North	SU 765042	50° 50'	1 m	100
Prinsted South	SU 764042	50° 50'	1 m	150

Table 2.2. The nearest weather stations to the various field sites. 'Distance' gives the distance between the field site and the weather station in miles; 'altitude' gives the difference in altitude (weather station - field site) used for correcting the mean monthly temperatures.

<u>Field site</u>	<u>Weather station</u>	<u>Location</u>	<u>Distance</u>	<u>Altitude</u>
Invergowrie	Leuchars	3468E 7209N	5 m	-9 m
Tentsmuir	Leuchars	3468E 7209N	3 m	1 m
Chatton	Tynemouth	4374E 5695N	44 m	-17 m
Newcastle	Tynemouth	4374E 5695N	15 m	22 m
Kildale	Durham	4267E 5415N	32 m	-78 m
Gibraltar Point	Gibraltar Point	On site	0 m	4 m
Swindon	Lyneham	4006E 1782 N	12 m	35 m
Prinsted	Hastings	5809E 1094N	67 m	44 m

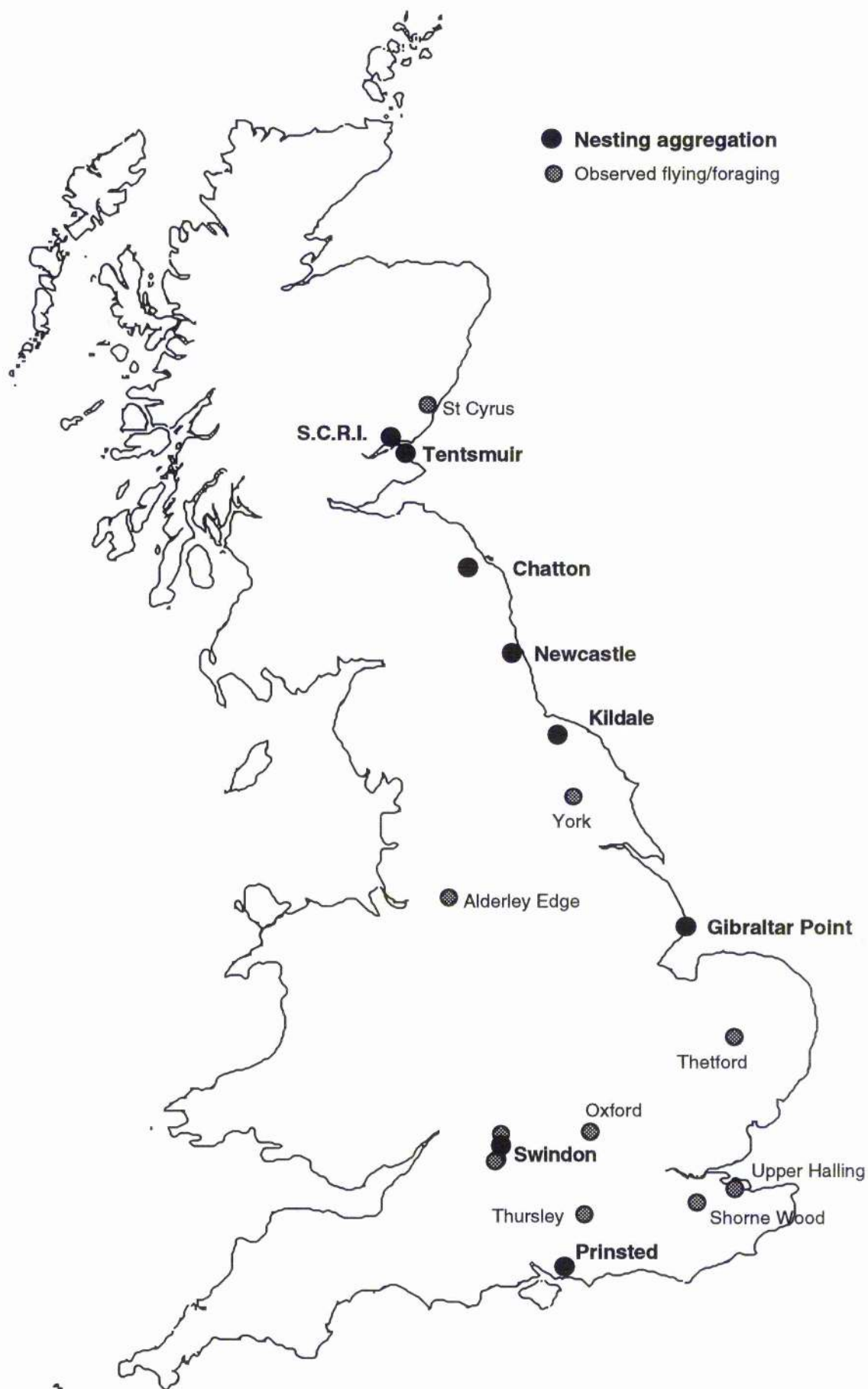


Fig. 2.1. UK map showing the locations of the field sites.

A.

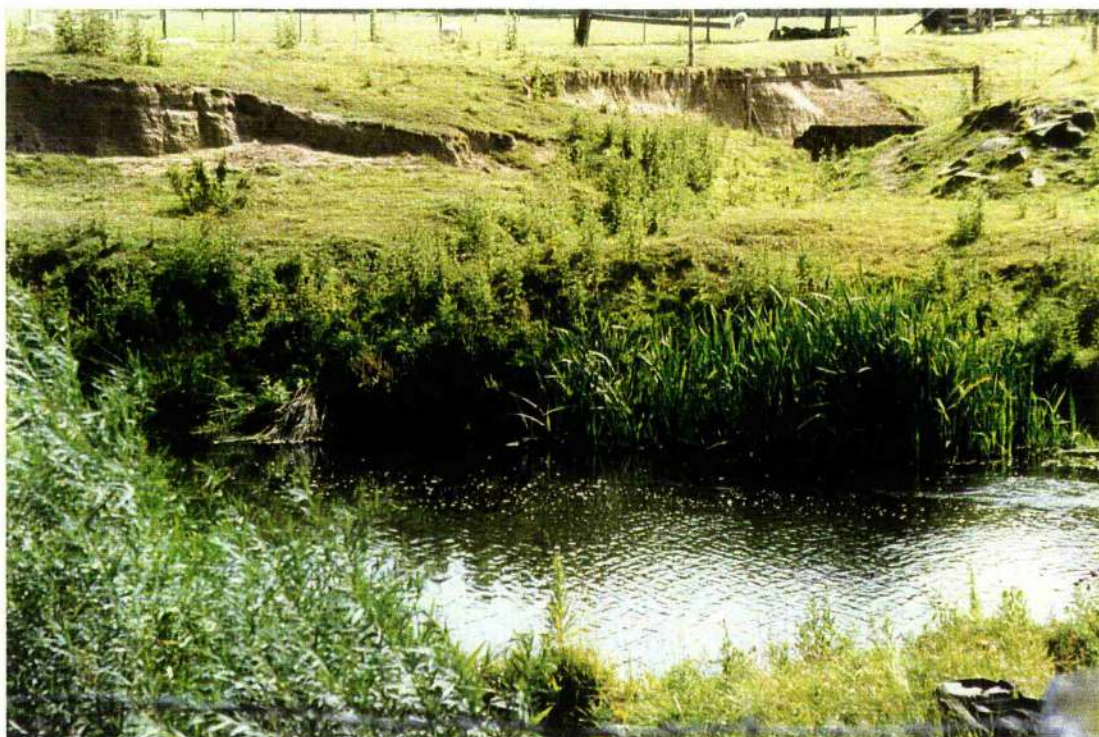


B.



Fig. 2.2. UK field sites. (a) Invergowrie (b) Tentsmuir East.

C.



D.

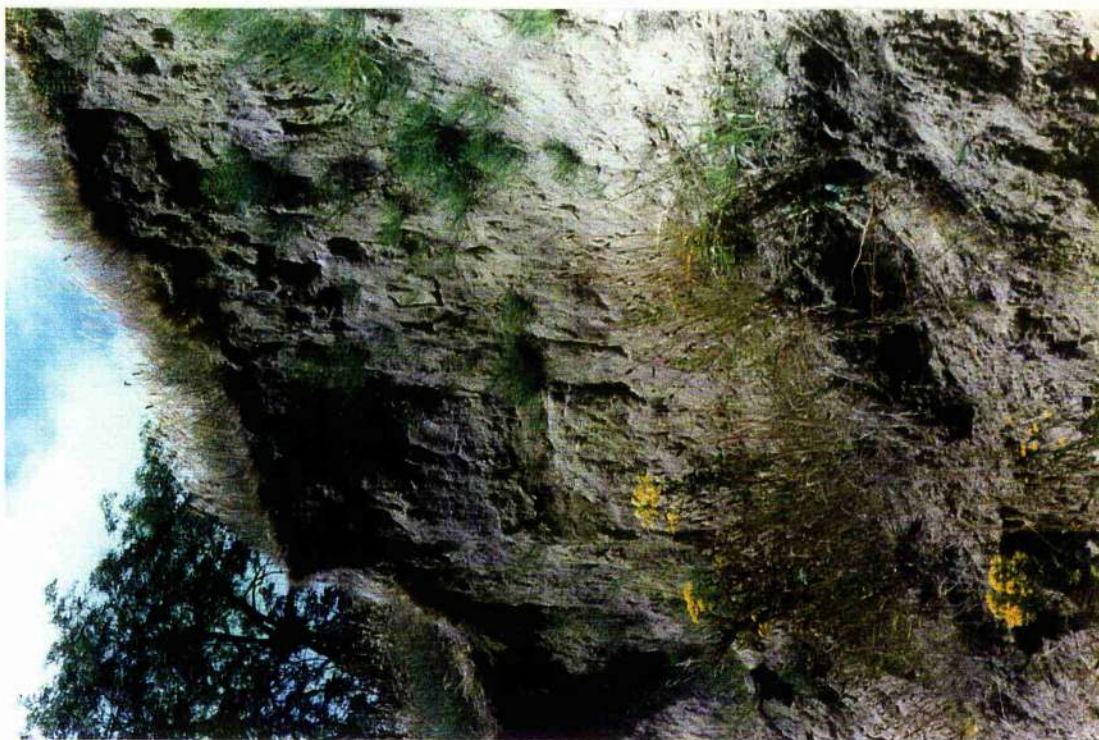


Fig. 2.2 (cont.). UK field sites. (c) Chatton (d) Newcastle.

E.



F.



Fig. 2.2 (cont.). UK field sites. (e) Kildale (f) Prinsted South.

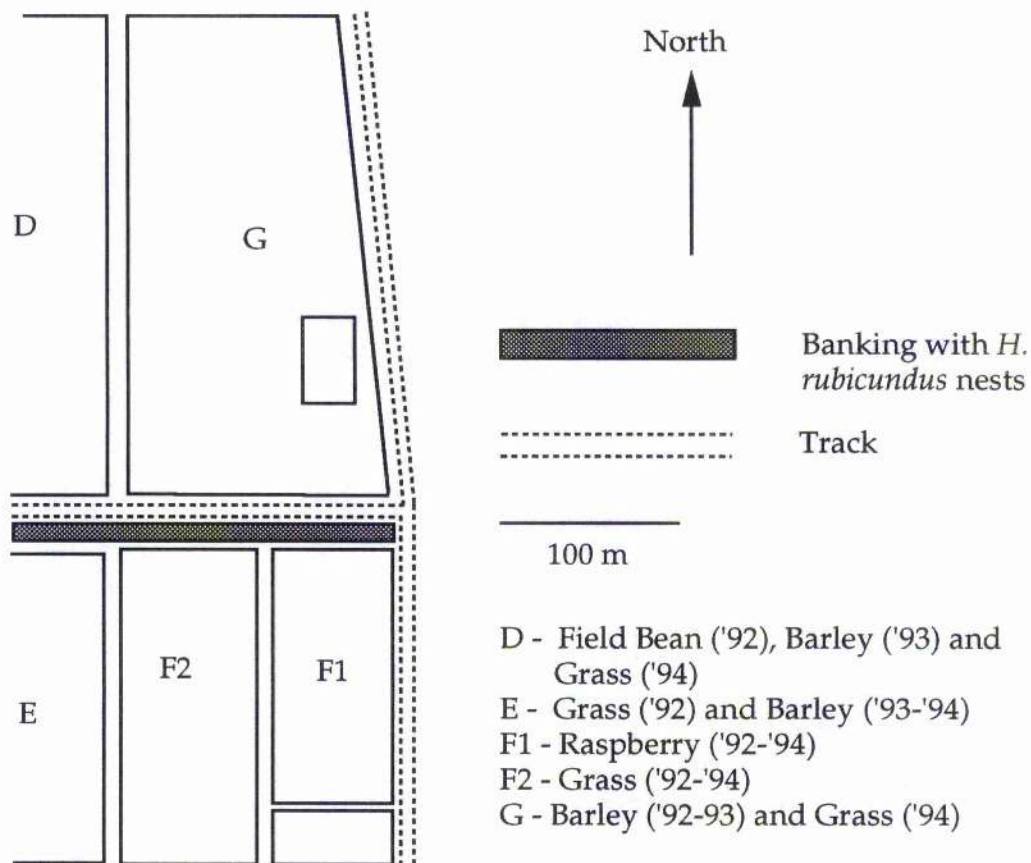


Fig. 2.3. Plan of part of the Scottish Crop Research Institute (SCRI) at Invergowrie containing the banking with *H. rubicundus* nests.

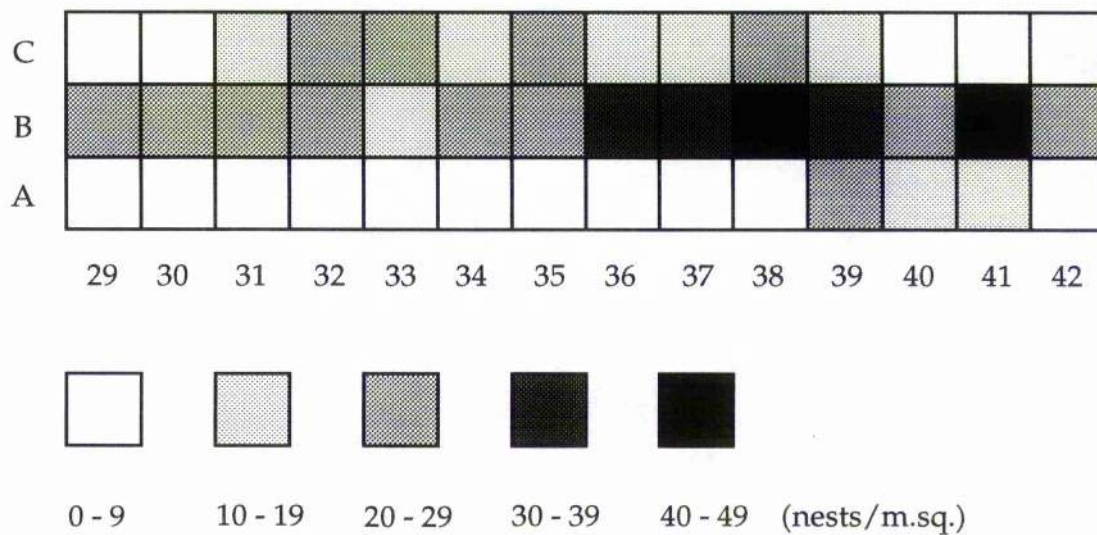
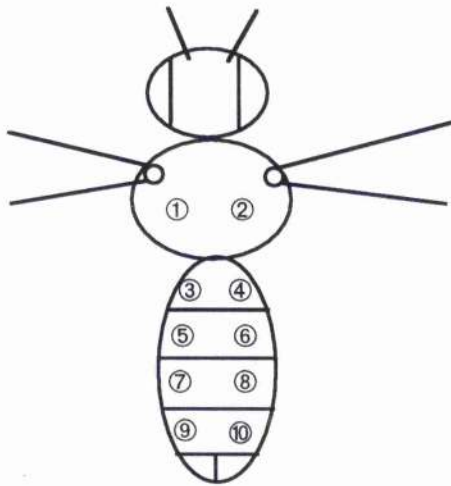


Fig 2.4. Density of nests in 1m square quadrats at the eastern end of the banking at Invergowrie (end of May 1993).



Fig. 2.5. Picture showing the use of an emergence trap to catch bees exiting the nest.

A.



○ Individual paint marks

B.

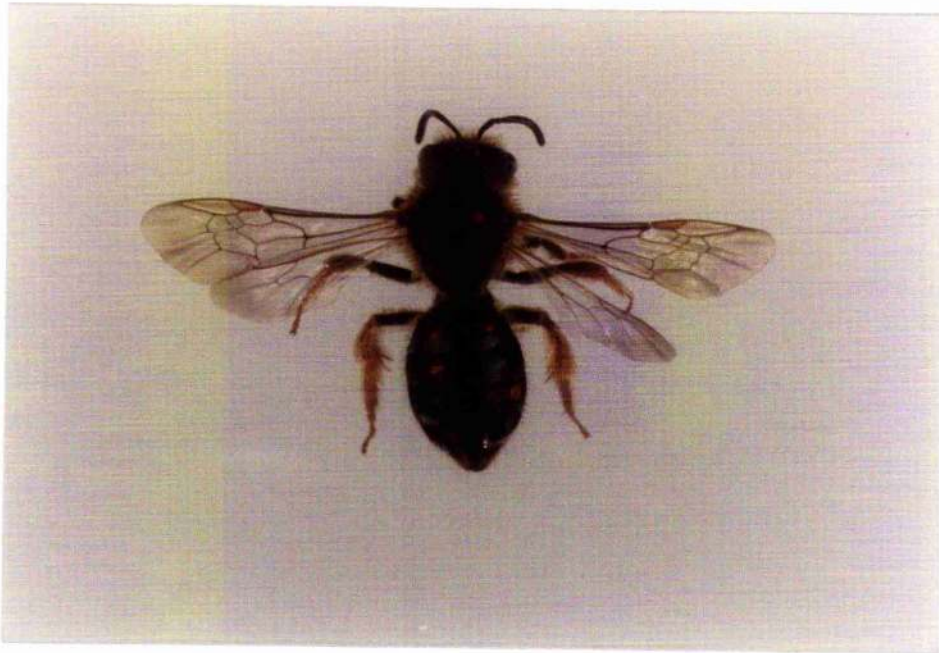


Fig. 2.6. (a) Diagrammatic representation showing possible marking positions on *H. rubicundus*. (b) Marked female.

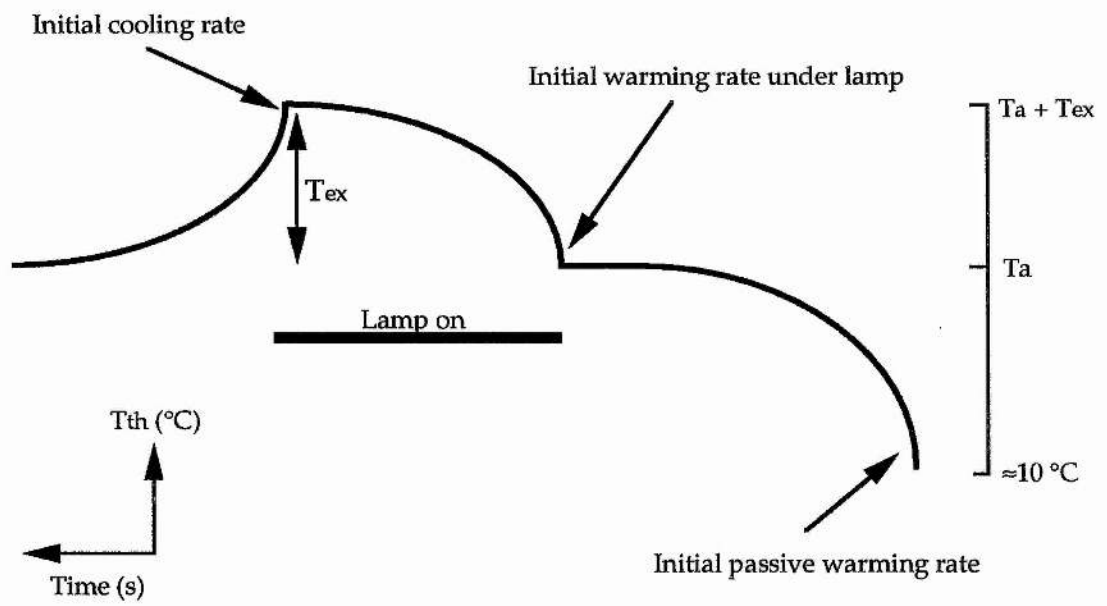


Fig. 2.7. Diagrammatic representation of a typical warming-cooling curve. T_{ex} is the temperature excess, and is the temperature the thorax (T_{th}) is above ambient temperature (T_a).

Chapter 3 General biology

3.1 Introduction

The UK has fifty species of Halictidae: 3 species of *Halictus*, 31 species of *Lasioglossum* and 16 species of *Sphecodes* (Betts 1986). There are in addition two species of *Dufourea*, but no sitings of these have been made since the 1950's. *Halictus rubicundus* is distributed throughout the British Isles, from the Channel islands north to the Scottish Highlands (Else in prep.). It is a very common species though usually only locally abundant when in close proximity to a nesting aggregation.

The overall geographic range is very large with *H. rubicundus* being found mainly in temperate regions of the Holarctic. In the Palaearctic it is distributed from Fennoscandia, south to Spain, Italy, Greece and Morocco. In North America it ranges from Alaska down to California and Florida (Knerer & Atwood 1962; Krombein *et al.* 1979).

Although widespread and generally abundant, very little work has focused on *H. rubicundus*. As a species it is often mentioned in brief as part of larger studies looking at behaviour and social organisation in the halictids as a whole (Batra 1968; Roberts 1973; Michener 1974; Barrows 1976a; Batra 1978).

More recently Packer and Owen (1989b) have looked at allozyme variation in *H. rubicundus*. Using 48 loci for 37 enzymes (with an average of 38 bees per locus), an expected heterozygosity of 0.038 ± 0.018 was obtained. This is well within the range found for other primitively eusocial bees (0.007 - 0.078). Using data from other populations it was concluded that the European and North American populations were quite distinct, with a Nei's genetic similarity (I) of only 0.764

(the two North American populations had a similarity of 0.934). For the same comparison, Nei's genetic distance (D) was 0.270 between the N. American and European populations and 0.068 within N. America; these values are consistent with interspecific differentiation found in other Hymenoptera. Therefore *H. rubicundus* is either unusual among Hymenoptera in exhibiting a high degree of population differentiation or it does really represent a species complex.

In a five year study of a large nesting aggregation in New York City, Yanega (1988, 1989, 1992 & 1993) gives a very detailed account of the social organisation of *H. rubicundus*. This population contained a mixture of solitary (similar to UK populations) and primitively eusocial nests (described below).

Fertilised females from the previous year returned to the site to found nests in the spring. Two female castes were produced in the first brood, gynes and non-gynes, together with a small number of males. Castes in this brood differentiated within the first few days of adult life, with gynes promptly leaving to overwinter (Yanega 1988). Non-gynes remained as workers and had the potential to become replacement queens; and it was found that the larger individuals were more dominant (Yanega 1989).

Gynes were significantly larger than non-gynes and this was largely due to the lateness of their emergence. The cue for caste determination was suggested to be promptness of mating after emergence, which in turn was dependent upon male abundance. Late emerging females would be more likely to be quickly mated as male emergence progressed through the season (Yanega 1992). Most of the workers were eventually mated, and there was some egg laying by workers as they aided the colony queen in the production of the second brood. This brood contained roughly equal numbers of females and males, with all the females being promptly mated and leaving the nest to overwinter elsewhere.

The proportion of males within a brood was correlated with ambient temperature and photoperiod, such that more males were produced when it was warmer. The more male biased the sex ratio was, the smaller the proportion of social nests there were in the population. Yanega (1993) suggests that abiotic environmental conditions during brood production may influence the demography of a population and hence the degree of sociality that is expressed. This phenomenon would then be a mechanism by which social behaviour, through caste determination, could 'fine-tune' social organisation to the characteristics of the environment (section 6.5).

3.2 Phenology

H. rubicundus is typical of British halictids, having a flight period of April through to September (Figure 3.1). There is some degree of asynchrony between individual nesting cycles and there can be a difference of up to three weeks in the emergence of the first females between nests. At Invergowrie females emerge from their hibernacula (overwintering burrows) during the last two weeks of April. In southern UK populations, emergence may be as early as the start of April and exceptionally even late March (Else, in prep.). Extensive excavation of the banking at Invergowrie revealed a few hibernaculae within the nest-site, but not enough to account for the population size; thus the majority of hibernacula must be sited elsewhere (i.e. in unknown location). The following descriptions of phenology refer to the observations at the Invergowrie field site based on 1992 to 1994.

Newly emerged female foundresses search for suitable sites for nesting and begin to dig a burrow. Excavation continues through the season as new cells are added and the nest is extended. Once a cell has been excavated it is completely

provisioned with pollen and nectar to form a pollen ball, and an egg is oviposited before the next cell started. The first cell is usually completely excavated and provisioned by the end of May, which is therefore the time the first eggs are laid. By late June most nests have 2 to 5 cells with pollen balls and eggs/larvae. By the end of June some the earliest cells contain prepupae or pupae; and adults begin to emerge soon after. This puts the developmental time from egg to adult at about 3 to 4 weeks (subject to prevailing microclimatic conditions).

The pollen ball is positioned in the centre of the cell and is a sphere of diameter 3.51 ± 0.06 (12) mm. The eggs are pearly white in colour and 2.69 ± 0.05 (7) mm long by 0.98 ± 0.06 (7) mm wide. They are laid on the dorsal surface of the pollen mass; and being gently arched, only the very tips of the egg touch the pollen mass (Figure 3.4b). The egg usually hatches a few days after oviposition and the young larva then begins to feed (Figure 3.2a); the larva probably moults five times during its development (Michener & Bennett 1977). Faecal pellets are present at the posterior end of the cell by the time the larva has entered the prepupal stage (Figure 3.2b) and feeding has finished. The pupa (Figure 3.2c) shows the basic external structures of the adult and there is a progressive darkening as pigmentation is laid down. This starts with the eyes changing from white to pink to brown to black, followed by a darkening of the whole body. Finally the pupal cuticle is shed and the adult emerges.

The brood begins to emerge around mid June and this continues through until mid August. Throughout this period the foundress is continuing to dig and provision new cells. Emergence is slightly protogynous with females emerging just before males. Using data from marked individuals caught in emergence traps over the season it was possible to calculate the number of surviving offspring per nest. The mean number of offspring per brood is 3.8 ± 0.3 (19) with 2.2 ± 0.2 (19) females and 1.7 ± 0.1 males (19). The newly emerged females are quickly mated,

by the males present, and then leave to overwinter in hibernacula until the following spring. In late August the foundresses numbers decline and by the end of September only a few males remain at the nest-site.

The seasonal cycle shown by *H. rubicundus* at Invergowrie is typical of that of a solitary bee. In Devon it has been reported that this species is double brooded in some seasons and may be primitively eusocial (Else, pers. comm.). Similarly, in North America, Yanega (1988) found *H. rubicundus* to have mixed populations exhibiting both solitary and primitively eusocial lifestyles. It follows from the 'fine-tuning' hypothesis (Yanega 1993) that in the cool temperate climate of Scotland and England populations would be expected to be solitary, and in the warmer conditions occasionally found in the south of England, *H. rubicundus* might be facultatively primitively eusocial. This social structure would probably be attainable in more northerly UK sites if the flight season and developmental times were not so constrained by the cooler weather.

Excavation of 4 nests at Kildale revealed that many more brood cells are present than at Invergowrie. A typical nest in mid July had 8 cells (5 pupae, 1 prepupa, 1 large larva still feeding on the remains of the pollen mass and 1 empty cell with faecal pellets present). It was estimated that about a third of the pupae were male and two thirds female, thus indicating that the sex ratio may be more female biased in Kildale.

Batra (1968) recorded the nest behaviour of North American *H. rubicundus* females which had nested between two sheets of acrylic plastic. It was observed that completed cells were left unsealed and frequently inspected by adult females. In one nest, unrelated females of the same generation co-operated in cell construction and provisioning; and several cells were completely stocked before the first egg was laid. This is not unusual behaviour for other primitively eusocial species of

Halictinae, but it may be important that the nesting conditions were very artificial in this study.

3.3 Diurnal nest cycle

Three typical nest cycles are given in Figure 3.3. Females leave the nest around mid morning and the first foraging trip(s) are often made without the collection of pollen. Later trips do however involve pollen collection, although this tends to be less so at the end of the day again, when presumably nectar alone is collected (see section 6.1). Activity is largely influenced by ambient temperature and this will be discussed later in section 4.4. Digging occurs toward the end of the day, typically after 14:00; but it may begin much earlier if weather conditions prohibit foraging. Digging was scored when loose soil was ejected from the burrow entrance; whether the excavation was of new cells or an extension of the main burrow it was impossible to tell.

3.4 Nest architecture

Females generally choose to site nests on sloping ground where the soil is exposed; the specific preferences are discussed in chapter 5. The nest structure is characteristic of small halictine bees, with a vertical main burrow with several individual cells located along its length (Sakagami & Michener 1962). A diagrammatic representation of a typical *H. rubicundus* nest, excavated on 27.6.94, is given in Figure 3.4a. Details of the form, size and numbers of various structures are outlined below for nests at Invergowrie. A few additional notes have been added where excavations at other sites have revealed interesting differences. The terminology used is in accordance with that of Sakagami and Michener (1962) in their review 'The nest architecture of the sweat bees'; according to this work the nests are classified as subtype IIIb (with the formula: O (Ls Ch)ⁿ B; where 'O'

indicates a main burrow with short laterals (Ls) connecting horizontal cells (Ch) and the presence of a lower blind burrow (B)).

3.4.1 Surface features

On the surface there is normally a tumulus of excavated soil (mean diameter = 19.7 ± 1.0 mm (13)) with the nest entrance at the centre. This structure is absent when nests are sited on steep bankings, or may be dispersed by wind and rain. The burrow is constricted at the entrance (mean diameter = 4.9 ± 0.2 mm (19) which is significantly smaller (two sample t test: $T = -3.54$ d.f. = 23, $p = 0.0017$) than the main burrow (mean diameter = 6.1 ± 0.3 mm (10)).

Constriction of the nest entrance is a predominant, if not universal, characteristic of halictine nests (Michener 1974), and has probably evolved in connection with the guarding habits of this group. In many species females will guard by blocking the entrance with their heads; this is especially true of the more social species where guarding is shared between several females. Roberts (1973) reports guarding behaviour by *H. rubicundus* females in primitively eusocial nests in Nova Scotia. At Invergowrie, short periods of guarding behaviour are demonstrated by *H. rubicundus* females in between foraging trips and this may help to prevent parasitic Diptera entering the nest (see section 3.8.1). However, if disturbed by a larger object (another bee or human observer for instance) the female will quickly retreat down the burrow. Unlike many closely related species there is no blocking of the entrance with the abdomen upon disturbance (Michener 1974). In general, though, the nest remains unguarded as the female is out foraging or is involved in cell construction and provisioning.

There may be another advantage to having a constricted entrance, namely that of reducing the risk of water logging. A small diameter hole will aid the flow of run-

off water in passing over rather than down into the burrow; this being especially true of nests in steeper sloping ground. This may, however, just be an incidental advantage arising from the primary guarding function of the constriction.

Narrowing of the entrance is accomplished by the female carrying soil from inside the burrow and tamping it into place with the apex of her pygidium. This is then smoothed with the mouthparts with the possible application of a salivary substance. The entrance can be completely sealed by continuing the process; this occurs in about a tenth of all nests at the end of the foraging cycle and also occasionally during the day just before heavy rainfall.

3.4.2 Main burrow

At Invergowrie and the other relatively flat sites, the main burrow generally runs straight down from the entrance in a vertical plane; the route may sometimes be circuitous to avoid obstructions such as stones. When nests are in near vertical bankings (for instance Chatton and Kildale) the burrow will go down into the banking at approximately 45° from vertical. At Invergowrie the mean burrow length in a mature nest (week 12 onwards) is 122.8 ± 9.2 mm (8). The length of the burrow increases through the season as the female excavates more cells (Fig. 3.5a) and the final depth seems to be determined by the change of substrate in the banking at these depths. The top soil is very sandy (see section 5.2.4), but below 100 mm or so the next soil layer has a much higher clay content and is generally water logged. The mean burrow diameter (6.1 ± 0.3 mm (10) - see above) remains constant throughout its length.

As is usual for halictine nests the burrow has a blind ending which is a length of tunnel below the deepest nest cell (mean length = 50.7 ± 33 mm (13)). Rather than deepen the burrow through the season just enough to allow the construction of

the next cell, *H. rubicundus* extends the burrow well beyond the brood cells. It has been suggested that this blind ending may serve some sort of homeostatic function to drain off excess water (O'Toole & Raw 1991), or even to elevate moisture levels in drier periods (Sakagami and Michener 1962); though there is no direct evidence for these, they both seem plausible. The blind ending also serves as a refuge if the nest is entered by a predator and is perhaps used as a source of soil for cell and burrow linings.

The walls of the burrow are smoothed with fine particulate matter but are not obviously impregnated with any secretion. Occasionally the main burrow will have a side branch ($2/47$ nests; lengths 27 and 37 mm); these go vertically downwards and may be where the original burrow was obstructed by a stone.

3.4.3 Brood cells and laterals

The number of brood cells increases steadily through the season (Fig 3.5b) and the mean number of cells in a mature nest is 4.9 ± 0.4 (18) with a mean cell depth of 49.1 ± 2.4 mm (86). The mean number of offspring observed in section 3.2 was 3.8 which is less than the number of cells in the nest; and this difference is accounted for by immature mortality due to parasitic diptera (section 3.8.1). The first cells within a nest are constructed close to the surface (mean depth = 31.8 ± 1.7 mm (33)) with subsequent cells being added lower down the main burrow as the season progresses. As expected, the mean depth of the deepest cell increases with time as the burrow is extended (Fig 3.6a). Cells are first sited close to the surface as this is the only place initially available; this results in shortened development times due to elevated soil temperatures from surface heating, and so may be particularly important at the beginning of the season when the ambient air is cooler.

The brood cells may be sited at any orientation around the main burrow and there is no preference shown at Invergowrie (Fig 3.6b: Rayleigh's test: $z = 0.6165$, $n = 78$, $p > 0.5$). The shape of the cell is typical of halictids, being a bilaterally symmetrical elongate oval with a constricted entrance (Fig. 3.4b). The mean length is 12.0 ± 0.3 mm (34) and the mean diameter is 6.5 ± 0.2 mm (41).

In the early stages of construction an oval cavity is excavated in the approximate dimensions of the final cell and possesses sides of rough particulate matter. Next a lining of about a millimetre thickness is added and this consists of fine clay which is worked into an extremely smooth covering. Finally, a wax like material is added to this lining and is presumed to be a mixture of macrocyclic lactones from the Dufour's glands as is found in other halictid bees (O'Toole & Raw 1991). It is a dark grey substance which seeps into the cell wall for a fraction of a millimetre or so. This coating serves two functions: firstly to control water balance by preventing dehydration or water logging; and secondly to inhibit fungal growth (Cane 1983a). The cell is closed with loose soil particles in the form a rough seal which is free of any Dufour's gland secretion.

H. rubicundus, with many other halictid species, shares the characteristic of brood cells being distributed along the upper part of the main burrow. Many other halictids are described as having the cells concentrated in a limited space and surrounded by a cavity. This is comprehensively reviewed by Sakagami & Michener (1962), and discussed with respect to possible homeostatic roles. There appears to be little relationship between complexity of nest structure and level of sociality expressed within the Halictidae.

The brood cells are connected to the main burrow by short laterals; these are narrower than the main burrow (mean diameter = 4.0 ± 0.2 mm (16)) and run at 90° to it. The mean length of the laterals is 12.0 ± 0.3 mm (34) but this varies with

the density of the surrounding nests. As local nest density increases, mean lateral length decreases (Fig 3.7: $y = 13.9 - 0.271x$, $r^2 = 0.938$, d.f. = 3, $p = 0.007$). This probably arises from the necessity to construct more compact nests in areas of higher nest density where the problems of adjacent nests collapsing into each other are greatest (see section 5.2.1). When a brood cell is completed the laterals are usually filled with loose earth.

3.5 Foraging

3.5.1 Pollen sources

H. rubicundus is polylectic (collects pollen from a wide range of plant species); however during the main provisioning phase (May, June and July) females tend to concentrate on locally abundant pollen sources and switch between these as pollen availability changes. Several flowering plants have records for visits by *H. rubicundus* (Table 3.1).

Females alone collect pollen and carry it on the femur and tibia of their hind legs. Sometimes the load may be much larger and extend to cover all the abdominal sternites and tergites as well. The mean pollen mass carried by a female is 1.7 ± 0.2 mg (8) and is usually of mixed species. For eight individuals sampled between early May and mid July the mean number of morphologically distinct pollen types carried by a returning female was 2.8 ± 0.4 (8) with a range of 1 to 4.

Oilseed rape (*Brassica napus*) is a locally abundant crop around Invergowrie. It flowers from early April until late July, but pollen is available for only part of this period. Assuming that the level of airborne pollen is a good indicator of pollen availability from *B. napus*, then the window for pollen collection by *H. rubicundus* is from late April until early June (weeks 2 to 8 in Figure 3.8). Flowers open at

about 09:00, with the peak period of pollen availability being 11:00 to 13:00; flowers then close by 20:00 (M J Wilkinson pers. comm.). This timing coincides with the foraging patterns displayed by *H. rubicundus* described in section 3.3. Of the four pollen loads examined during the weeks of peak pollen availability (from females returning to the nest after foraging) the percentages of *B. napus* pollen carried were approximately 100%, 80%, 20% and 10% (Table 3.2a). The nearest *B. napus* cultivar is approximately 1.5 km from the S.C.R.I. at Invergowrie (A M Timmons & M J Wilkinson, pers. comm.); therefore females use this pollen source in apparent preference to other sources much closer to the nest-site.

As the season progresses and the *B. napus* pollen is no longer available, other flowering plants are visited (Table 3.1). *H. rubicundus* females have also been observed visiting cultivated raspberry (*Rubus idaeus*), brambles (*Rubus fruticosus*), gorse (*Ulex* sp.), and dandelion (*Taraxacum* sp.). Overall then, females are able to collect a very wide range of pollen types, and at Invergowrie (and possibly other sites) foraging is concentrated on oilseed rape and when this is finished a broad range of other sources are then utilised. In most parts of its range *H. rubicundus* is reasonably common and presumably a major pollinator, especially of many Compositae (Table 3.1); similarly Michener (1977) suggests that *H. ligatus* is an important pollinator of Compositae in its US home range.

3.5.2 Cell provisioning

During the main provisioning phase females make a mean of 7.1 ± 0.4 (12) foraging trips per day in good weather, of which a mean of 5.1 ± 0.3 (12) will be for pollen (and probably nectar too).

An estimation of the number of pollen foraging trips needed to provision one cell completely can be made from the mean mass of a full pollen ball (dried) and the

mean mass of pollen carried by a returning female. Pollen balls weighed 18.5 ± 1.2 mg (10) and a female carried 1.7 ± 0.2 mg (8), thus giving an average of 11 pollen trips per provisioned cell.

Table 3.2b summarises the pollen composition of completed cells. The mean number of pollen types found was 4.6 ± 0.4 (9) with a range of 3 to 7. The mean percentage of *B. napus* pollen incorporated (in terms of grain numbers) was estimated to be 41.7 ± 7.7 % (9).

3.5.3 Forage trip duration

The length of each pollen foraging trip is not correlated with time of day ($y = -0.730x + 31.133$, $r^2 = 0.003$, d.f. = 109, $p = 0.237$). It is however significantly correlated with ambient temperature (Figure 3.9a: $y = -0.947x + 45.163$, $r^2 = 0.047$, d.f. = 108, $p = 0.023$), such that trips become increasingly short as ambient temperature rises. Similarly there is no relationship between time spent within the nest between foraging bouts and time of day ($y = -1.656x + 43.773$, $r^2 = 0.013$, d.f. = 106, $p = 0.204$). Further there is no significant correlation between nest time and ambient temperature (Figure 3.9b: $-0.493x + 35.621$, $r^2 = 0.014$, d.f. = 122, $p = 0.196$).

The minimum time required for foraging (assuming constant flight distances and pollen handling times) will be a function of ambient temperature (see section 4.5.4E) and therefore it would be expected that there is a correlation between these two parameters. Time spent within the nest, however, is going to be dependent upon the activity being undertaken; if a pollen load is simply being deposited in a cell then the 'in nest' duration will be short. However cell construction and pollen mass sculpting will take much longer and thus give longer within nest times. Another factor that accounts for variation in time spent inside is the presence of

parasitic Diptera at the nest entrance, which will often delay the female bee's exit (see section 3.8.1). It is therefore not surprising that nest duration varies independently of ambient temperature; undoubtedly though, specific activities within the nest will be dependent upon temperature. The influence of microclimate on various activities will be discussed later in more detail in section 4.

3.6 Male activity

Males begin to emerge in late June and this continues through until mid August. Males can still be seen flying until late September, thus indicating a life span of between six and eight weeks.

Patrolling by halictid bees is defined by Barrows (1976a) as repeated flying by males (that are not feeding) among particular landmarks which may be rendezvous places (locations where males are likely to find mates), with flights usually confined to particular topographical areas. *H. rubicundus* males certainly exhibited this kind of behaviour and this form is more specifically known as 'non-sequential' patrolling. Flight is usually rapid and in a characteristic zig-zag fashion just a few centimetres above the banking surface. Males fly in irregular sequences among sets of landmarks on the banking (nest markers, prominent stones etc.), and do not fly in any particularly well defined routes in any particular sequence. Patrolling paths continually interweave with those of other conspecifics and males do not defend these routes against other males.

Patrolling is observable from the end of June until September on the banking; but it is unlikely to occur at the female feeding sites as is common in other species. This is because newly emerged females are only present at the banking for a few days before they leave to overwinter; during this time they will make very few

foraging flights, and the area of flowers available is so vast at Invergowrie that males would have little chance of securing matings at sites away from the banking.

In their patrol flights males commonly contact females and occasionally secure matings. A male will closely follow a flying female (in 1 cm oscillations above and behind her) and will immediately pounce if she lands on the ground; the majority of females, however, return straight to their nests and enter the burrows before males have any opportunity to mate.

Males do not distinguish between newly emerged virgin females and older foundresses carrying pollen (or indeed between these and small pebbles and other males) when pouncing on individuals on the ground. However, anything that is not a female *H. rubicundus* is quickly ignored. Males will very quickly begin to copulate with receptive virgin females, as the pair is quickly discovered and joined by other patrolling males. Up to ten males may be vying for position around a female once on the ground. Copulation is short, lasting 43.7 ± 6.7 (6) seconds, before the female flies off. It is unclear whether females mate multiply (as with *Halictus ligatus*, Packer 1986a), as matings were not commonly observed and a marked female was never seen to mate more than once.

Males are generally actively searching the nest-site up to an hour before the first females appear in the morning, and will continue to patrol after the majority of females have finished foraging. Even though the probability of encountering a female will be relatively low during these periods it appears that the male searching strategy is worthwhile. The remote chance of a mating opportunity does not seem to be offset by any particular disadvantage such as increased exposure to predators. The times and ambient conditions during which males are active are discussed in detail in the next chapter.

During cooler conditions, and outside normal activity times, males will remain in their natal burrows, abandoned burrows or any other suitably sized hole in the ground. At low ambient temperatures when females are still actively foraging males can be observed walking on the banking surface as they are unable to fly, and they are presumably still seeking matings. Often males are found sleeping in groups; it is unclear if there is any adaptive advantage to this, but it is a common trait in many halictid species (Barrows 1976a).

3.7 Size

3.7.1 Head width and mass

For a given morphotype, length can be expressed as a fractional power of mass (volume) such that length is proportional to $\text{mass}^{1/3}$. For *H. rubicundus*, when both sexes are considered together, head width is very highly correlated with the cube root of the live body mass ($y = 6.03x - 8.68$, $r^2 = 0.995$, d.f. = 90, $p < 0.001$). This suggests that males and females share very similar morphotypes and may be considered together for certain analyses.

However, for the relatively small size ranges found within each sex, it is convenient to use a linear measurement of size (head width) as this is easily measured in the field and also highly correlated with mass. Head width has been used as a good indicator of size for several halictid studies (e.g. Abrams & Eickwort 1980; Packer & Knerer 1986; Wcislo *et al.* 1993). The same relationship is found for *H. rubicundus*; within a population head width is significantly correlated with wet mass for both sexes (Figure 3.10a: females, $y = 0.014x + 2.209$, $r^2 = 0.500$, d.f. = 38, $p < 0.001$; males, $y = 0.023x + 1.972$, $r^2 = 0.353$, d.f. = 46, $p < 0.001$). In addition these two size measurements are significantly correlated for the seven

populations across the UK (Figure 3.10b: $y = 0.25x + 1.763$, $r^2 = 0.854$, d.f. = 5, $p = 0.003$).

Figure 3.11a gives a frequency distribution of head widths for *H. rubicundus* at Invergowrie: the mean for females is 2.72 ± 0.01 (159) mm and the mean for males is 2.36 ± 0.01 (48) mm. The difference in mean head width between the sexes was very significant (Figure 3.12b. t test: $T = 19.07$, d.f. = 144, $p < 0.001$). The mean mass of females was 30.5 ± 1.1 mg (42) and the mean mass for males was 16.9 ± 0.4 mg (47).

3.7.2 Size and latitude

Small animals have relatively high surface area to volume ratios and consequently have high rates of heat exchange with the environment. Bergmann's rule states that endothermic animals in cooler climates will tend to be larger than their relatives in warmer climates. An ectotherm's activity depends almost entirely upon ambient temperature, and so it would be expected that body size would be closely linked to prevalent environmental conditions (see section 4.5). By extension of Bergmann's rule we would expect that populations inhabiting cooler climes would have larger body masses than those in warmer climes. This has been shown to be true for feral honeybees in California (Daly *et al.* 1991).

Mean head width of *H. rubicundus* varies by nearly 1 mm between populations across the UK, with the northern populations generally containing the larger individuals. However, the relationship between latitude and head width is not significant (Figure 3.12a: $y = 0.001x + 2.112$, $r^2 = 0.340$, d.f. = 5, $p = 0.169$). If the mean monthly minimum temperatures through the season are considered though (see section 2.5.1), then there is a negative correlation with head width (Figure 3.12b: $y = -0.154x + 4.073$, $r^2 = 0.628$, d.f. = 5, $p = 0.036$). Variation in temperature

can thus explain over 60% of the variation in size of the populations in the UK. There will be selection for larger females in cooler areas as this will allow individuals to forage earlier in the season, and for longer periods and when the weather is cooler (see section 4.5).

3.7.3 Size variation through the season

Females begin to arrive at the banking in Invergowrie during week 1 (the end of April), with their numbers steadily increasing up to weeks 5 and 6 (Figure 3.13). The mean head width of female foundresses searching the nest-site in weeks 4 and 6 is 2.78 ± 0.03 (32) mm. The mean head width for all females (both foundresses and newly emerged females) for weeks 9 to 20 is 2.67 ± 0.02 (96) mm, and there is no significant difference between the weekly means for this period (oneway ANOVA: $F = 0.68$, d.f. = 95, $p = 0.740$). However the 'foundress only' mean was significantly greater than the female mean for the rest of the season (t test: $T = 3.06$, d.f. = 53, $p = 0.0035$).

There are two possible explanations for the difference in these two means. Firstly, the foundresses arriving at the beginning of the season are maybe the larger ones since these can emerge, fly and search at lower mean ambient temperatures than smaller individuals can (see section 4.5). As the season progresses and smaller foundresses arrive, so the weekly mean head width decreases until there is no observable difference between weeks. Secondly, there may be some degree of size dependent mortality in the overwintering stage, such that larger females are more likely to survive and return the following year to found a new nest than smaller individuals.

Males start to emerge in week 12 and there is no significant difference in head widths between the weeks (oneway ANOVA: $F = 0.93$, d.f. = 75, $p = 0.479$), so that the overall mean head width for the male flight period was 2.36 ± 0.01 (76) mm.

3.8 Parasites and parasitoids associated with *H. rubicundus*

Several very different organisms have been found associated with halictids (Table 3.3). It is very likely that *H. rubicundus* has many of these organisms associated with it, although these were not specifically sought in this study. In the UK these include two species of kleptoparasitic bee (cuckoo bee) and a parasitic fly.

3.8.1 Parasitic Diptera

In northwestern America, Roberts (1973) reported a *Leucophora* sp. parasitising *H. rubicundus* and Knerer & Atwood (1967) described *H. rubicundus* and *Dialictus lineatulus* suffering heavy mortality due to the impact of *L. unistriata* and *L. johnsoni*. The latter species was also described associated with *Lasioglossum zephyrum* in Kansas (Batra 1965). In the UK another anthomyiid fly closely related to the North American species was observed following female bees to their nests at Invergowrie, Tentsmuir and Chatton. This was identified as *Leucophora grisella* (G Rotheray, Royal Museum of Scotland) which is a relatively common species throughout Northern Europe (Figure 3.14a).

Female *L. grisella* are seen flying at Invergowrie from late April until late June, which coincides with the hosts provisioning phase. These flies rest on the banking motionless, making the occasional short flight to another position. An approaching bee stimulates pursuit, with pollen laden females being followed very closely. A host will usually be followed by a single fly, although up to five flies have been seen tagging one individual. On a typical warm day (such as

13.6.94), 31.4 % of returning females ($n = 70$) were followed by at least one fly. The bees often attempt to shake off the pursuing Diptera by flying fast, close to the banking surface, and making several sharp turns, thus delaying entry into the nest. This is, however, seldom successful in dislodging the parasite(s); occasionally when one bee comes into close proximity with another, the fly will switch hosts (less than 5% of the time, pers. obs.).

When the bee finally returns to the nest, the fly will enter the nest immediately after the host (40.1 % of cases (22)) or remain at the entrance (Figure 3.14b) until the female next leaves (59.9 % of cases). The fly quickly enters the nest backwards and stays inside for a mean time of 31.0 ± 10.8 (6) seconds. The female Diptera oviposits a first instar larva into any cell that is open and contains some provisions. Many cells contain more than one parasitic larva; it is unclear, however, whether this is due to multiple oviposition by a single female or single oviposition by several females.

Excavations carried out for the period when *L. grisella* were present revealed that 65.0% of nests had been parasitised (Figure 3.15a); and for those nests that had been attacked, 29.1% of all the cells contained at least one diptera larva with a mean of 1.62 ± 0.17 (34) larvae per cell and a range of one to five (Figure 3.15b). Batra (1965) found that 10% of *Lasioglossum zephyrum* pollen balls had been parasitised by *L. johnsoni*; and Packer *et al.* (1989a) report that 0.0 % and 0.3 % of *Lasioglossum comagenense* and *Augochlorella striata* cells respectively, had Miltogrammine larvae present. The numbers of larvae present in a nest varies with local nest density and is discussed in section 5.2.5.

The parasitic larvae feed upon the pollen mass, quickly turning it into a liquid soup, so that the bee egg/young larva drowns or is soon outcompeted in terms of food utilisation, or may even be attacked and killed by the parasitic larvae. Once

feeding is complete the fly larvae then burrow into the soil surrounding the cell and pupate. They remain in this stage until the following spring, whereupon they emerge (presumably triggered by temperature changes) as adults and continue the life cycle. It is unclear at present when mating occurs and what the male life cycle involves.

The behaviour of *H. rubicundus* females is often modified in response to the presence of *L. grisella*. Often a female will delay exit from the nest if there are parasitic Diptera waiting at the entrance to the nest. The female bee will remain at the entrance in what appears to be some form of guarding behaviour. This seems effective since the flies are unable to enter; however as soon as the female returns inside the nest the flies may also follow. Sometimes the bee will immediately return to the surface and cause the intruder(s) to leave but in other cases, where the bee remains within the nest, there is ample time for the parasite(s) to oviposit. A typical example is that of the female bee Red 80 which made only three foraging trips on 10.6.94, whereas other females made many more. There were Diptera present around the vicinity of the nest entrance from 09:08 until 11:13; Red 80 failed to leave the nest when ambient conditions were suitable for foraging and instead remained at the entrance, blocking the entry of the two Diptera present. Three times Red 80 moved down into the nest and was instantly followed by the flies only to return to the surface after a few seconds (2, 5 and 4) and expel the parasites. When Red 80 did finally leave the nest at 11:13, two parasites entered and presumably oviposited.

At Invergowrie *L. grisella* exacts a very high toll upon the population of *H. rubicundus* and is the primary cause of juvenile mortality. For the three years of study it is not really possible to show definitively whether the population as a whole is declining due to the impact of the parasite, although this would seem likely with respect to the high parasite load present. The number of nests in the

quadrat of highest density (41B) for the years 1993, 1994 and 1995 was 47, 37 and 12 respectively. This decrease fits with the parasite argument but other factors (e.g. pathogen outbreak) can not be ruled out entirely. The decline and death of ground nesting hymenopteran aggregations is a well known phenomenon (Michener 1974).

3.8.2 Kleptoparasitic bees

Kleptoparasitic or cuckoo bees are essentially bees that lay their eggs in the nests of another species without contributing anything to the provisioning of their offspring. This habit is obviously a successful strategy as kleptoparasites account for some 19% of all known bee species (O'Toole & Raw 1991). This mode of reproduction must have evolved at least 15 times within the Apoidea and at least once within the Halictidae. Kleptoparasites are usually closely related to their host species (Roubik 1989); and for halictid bees the parasites are all from the genus *Sphecodes* and the majority of hosts are *Halictus* or *Lasioglossum* sp.

Cuckoo bees share several common characteristics that distinguish them from their hosts. Since they do not forage to provide food for their offspring they have lost the pollen carrying structures normally associated with their hosts, such as pollen scopae and corbiculae. They possess thick cuticles that protect them from attack by host bees, and often have oversized and sharp mandibles suited to fighting (Michener 1978). Female cuckoo bees are able to produce more oocytes than their hosts and at any one time their ovaries contain more mature oocytes (Iwata & Sakagami 1966; Alexander & Rozen 1987), although these are usually relatively smaller in size. These adaptations ensure that the kleptoparasite is able to exploit a host nest maximally once it is located.

The host-kleptoparasite relationships of various halictid species across Eurasia and North America have been described in some detail (Ordway 1964; Knerer & Atwood 1967; Eickwort & Eickwort; 1972; Michener 1974; Batra 1978; Roubik 1989; Westrich 1989). Within the UK two species of *Sphecodes* have been recorded parasitising various Halictidae. *Sphecodes gibbus* attacks the nests of *H. rubicundus* (Betts 1986; Else in prep.; pers. obs.) and also possibly *L. malacharum* (Else in prep.). The closely related *Sphecodes monilicornis* has been recorded with the following hosts: *H. rubicundus* (Betts 1986; Else in prep.; J. Field pers. comm., pers. obs.); *L. calceatum*, *L. xanthopum*, and *L. laticeps*, *L. malacharum* (Betts 1986; Else in prep.).

S. gibbus was observed searching the nest-sites at all of the field sites as far north as Chatton. *S. monilicornis* was however only identified at Newcastle, Gibraltar Point and Prinsted. Both species are fairly common throughout Europe and reach their northernmost limits of distribution in southern Scotland (*S. gibbus*) and central Scotland (*S. monilicornis*). The fact that both species are absent from any of the Scottish sites probably reflects the general tendency that near the edge of their ranges animals are rare and only locally abundant.

The flight season for females for the two *Sphecodes* sp. is late April until early September for the south of England and will be shorter for more northerly sites (Betts 1986). Both species share very similar life cycles and so will be described together with any differences being noted.

Overwintering females emerge in April and start searching for host nests with a characteristic low level zig-zagging flight. Any burrow-like openings in the ground are investigated (whether they are nests or not) by the females hovering over them in close proximity. Kleptoparasitic bees probably detect active nests using chemical cues emanating from the nest and the host (Roubik 1989); and once

a suitable nest has been located it is entered almost immediately. Female *Sphecodes* are quite aggressive (especially *S. monilicornis*) and will attack and kill any host bees encountered (Michener 1978).

In a primitively eusocial North American *H. rubicundus* nest in an observation tank, a female *Sphecodes* sp. was observed entering the nest (Batra 1978). The cuckoo bee was prevented from reaching the cells by the resident female filling in the burrow below the intruder. It was not stated, however, whether the intruder attacked the host or attempted to dig down to the brood cells.

Completed brood cells are then opened and the host eggs and young larvae killed before the cuckoo oviposits an egg on the pollen mass in an equivalent position to the host egg. The eggs are very similar to those of the host, being only a little shorter and straighter. Finally the cells are resealed, and the nest entrance may also be sealed after the kleptoparasite has left. It is unclear how many cells are parasitised by *S. monilicornis* and *S. gibbus* or how many nests through a season a single female will attack. This is largely because the sites with these two *Sphecodes* species present were unavailable for investigative excavations.

For *Sphecodes* sp. in general a female will remain in the nest overnight, or for several days, until complete oviposition is achieved (Knerer and Atwood 1967). The larvae are very similar in structure and behaviour to those of the host and presumably take the same amount of time to develop (Michener 1978). Emergence occurs between mid July and September with the females being quickly mated and then leaving to overwinter.

Since kleptoparasites are freed from the necessity to build and provision a nest, flower visitations will be usually for nectar collections with some pollen taken for an individual's own nutrition. *S. monilicornis* and *S. gibbus* have been observed

feeding upon a wide range of flowering plants (see table 3.1), and are also common visitors to *Heracleum* sp. (hogweed). This wide range of plant species is indicative of the long flight season of these bees as with the hosts.

Although it is not possible to quantify the true impact of these kleptoparasites on *H. rubicundus* aggregations, the overall effect is likely to be minimal. *Sphecodes* sp. were really only abundant at Chatton and Newcastle (pers. obs.); and the possible influence on the host species is discussed in section 5.3.4. In general however it appears that mortality due to kleptoparasitism at these sites is much less than the mortality due to *L. grisella* at Invergowrie.

3.9 Summary

H. rubicundus in the UK represents a typical example of a temperate species of halictid bee. Its seasonal cycle depends very much upon prevailing ambient conditions, as do the various daily activities of foraging, patrolling and nest provisioning (discussed in section 4.5).

It is a polylectic species with a long provisioning period and is able to utilise a wide range of pollen sources. Large distances may be covered, at Invergowrie, in order to obtain sufficient pollen of the required type to stock cells, even though other more local sources are available.

The nest architecture shows several features that allow brood development to take place in favourable conditions. These include the positioning of cells in the most suitable part of the available substrate, and the use of a blind burrow.

The social organisation is predominantly that of a solitary species. However *H. rubicundus* may be able to establish facultatively eusocial colonies in the UK when

not constrained by cooler climatic conditions, as can be seen in some populations in North America (discussed in chapter 6).

Across the UK there is a large variation in female size and this can largely be accounted for by the prevailing temperature conditions. The variation in mean minimum monthly air temperature explains some 62.8% of the variation in head width.

Associated with *H. rubicundus* at the Invergowrie site is the parasitic fly *Leucophora grisella*. From the exceptionally high brood mortality (29.1% of all cells parasitised), it is apparent that the bee population is under extreme pressure and the aggregation may even be dying off. Other sites are relatively free of this parasite, but some are instead attacked by kleptoparasitic bees of the genus *Sphecodes*.

It is estimated that, on average, 11 foraging trips are required to completely stock a single cell with pollen. If four offspring (mean of 2.2 females and 1.7 males per nest) are produced then a total of 44 trips will be necessary. On the most productive foraging days (i.e. those when ambient conditions allow the maximum number of trips to be undertaken) a mean of six pollen loads can be returned to the nest (section 3.3); thus a minimum of 8 full foraging days are needed to rear a brood. In addition, nearly 30 % of cells fail to produce offspring due to the presence of *Leucophora grisella* larvae (section 3.8.1); and these have also been stocked with pollen. A further 13 trips (equivalent to nearly 3 extra days) are needed thus giving a minimum of 11 full foraging days. This is an underestimate as it assumes every day is a 'highly productive foraging day' (a rarity at Invergowrie) and further does not include any cells that are partially provisioned and then abandoned. The period between the start and finish of provisioning is 80 days (section 3.1), and at least 25 % of these have weather entirely prohibiting

foraging and a majority of remaining days with much less than six trips per day (pers. obs.). It would appear then that offspring production is seriously limited by the periods of suitable weather for foraging and by parasite impact.

Overall then, many aspects of this bee's biology and activity patterns are profoundly influenced by the prevailing climate and microclimatic conditions. These are to be investigated in detail in the next chapter.

Table 3.1 Summary of flower records for *H. rubicundus* in Britain and Northern Europe. For the reference column: 'GRE' indicates Else, (in prep.); 'PW' indicates Westrich (1989) and 'SGP' indicates this author. For the flowering column the figures indicate the flowering period in months (Fitter & Fitter 1974). '+' indicates that *Sphecodes gibbus* or *Sphecodes monilicornis* has also been observed visiting this species.

<u>Plant species</u>	<u>Common Name</u>	<u>Reference</u>	<u>Flowering</u>
Campanulaceae:			
<i>Jasione montana</i> ⁺	sheepsbit scabious	PW	5-9
Compositae:			
<i>Achillea</i> sp. ⁺	yarrow	GRE	6-11
<i>Aster</i> sp.	Michaelmas daisy	GRE	8-11
<i>Centaurea</i> sp.	knapweed	GRE	6-9
<i>Centaurea jacea</i>	brown knapweed	PW	6-9
<i>Centaurea scabiosa</i>	greater knapwood	PW	6-9
<i>Cichorium intybus</i>	chicory	PW	6-9
<i>Cirsium arvense</i> ⁺	creeping thistle	PW, GRE	6-9
<i>Cirsium vulgare</i> ⁺	spear thistle	PW, GRE	7-9
<i>Hieracium pilosella</i> ⁺	hawkweed	PW	6-9
<i>Hypochoeris radicata</i>	common catsear	PW	7-10
<i>Leontodon</i> sp.	hawkbit	GRE	6-10
<i>Leontodon autumnalis</i>	Autumn hawkbit	PW	6-10
<i>Leucanthemum vulgare</i>	ox-eye daisy	PW	5-9
<i>Matricaria</i> sp.	pineapple mayweed	GRE	5-11
<i>Pulicaria dysenterica</i>	common fleabane	GRE	7-9
<i>Senecio jacobaea</i> ⁺	ragwort	GRE	6-11
<i>Senecio vernalis</i>	Oxford ragwort	PW	4-11
<i>Sonchus arvensis</i>	perennial sow-thistle	PW	7-9
<i>Tanacetum</i> sp.	tansy, feverfew	GRE	7-9
<i>Tanacetum vulgare</i>	tansy	PW	7-10
<i>Taraxacum</i> sp. ⁺	dandelion	GRE, SGP	4-6
<i>Taraxacum officinale</i> ⁺	dandelion	PW	4-6
Cistaceae:			
<i>Helianthemum chamaecistus</i>	rock-rose	GRE	5-9
Cruciferae:			
<i>Brassica napus</i>	rape	PW, SGP	5-9
<i>Sinapis alba</i>	charlock	PW	4-10
<i>Sinapis arvensis</i>	white charlock	PW	4-10
Dipsacaceae:			
<i>Scabiosa</i> sp.	scabious	GRE	6-10
<i>Succisa pratensis</i>	scabious	PW, GRE	6-10
Ericaceae:			
<i>Calluna vulgaris</i> ⁺	heather	GRE	7-9
Euphorbiaceae:			
<i>Euphorbia</i> sp. ⁺	spurge	GRE	5-9

Geraniaceae:			
<i>Geranium sylvaticum</i> [†]	wood cranesbill	PW	6-9
Leguminosae:			
<i>Melilotus alba</i>	white melilot	PW	7-10
<i>Trifolium repens</i>	white clover	PW	5-10
<i>Ulex</i> sp.	gorse	GRE, SGP	7-11
Menyanthaceae:			
<i>Menyanthes</i> sp.	bogbean	GRE	4-6
Ranunculaceae:			
<i>Ranunculus</i> sp.	buttercup	GRE	4-10
<i>Ranunculus acris</i>	meadow buttercup	PW	4-10
Rosaceae:			
<i>Malus domestica</i>	cultivated apple	PW	5
<i>Potentilla reptans</i> [†]	creeping cinquefoil	PW	5-9
<i>Prunus</i> sp.	blackthorn	GRE	3-5
<i>Pyrus communis</i>	cultivated pare	PW	4
<i>Rubus</i> sp.		GRE	
<i>Rubus fruticosus</i>	bramble	PW, SGP	5-11
<i>Rubus idaeus</i>	raspberry	SGP	5-8
Salicaceae:			
<i>Salix repens</i> [†]	creeping willow	PW, GRE	4-5
Umbellifereae:			
<i>Angelica</i> sp. [†]	Angelica	GRE	7-9
<i>Daucus carota</i> [†]	wild carrot	GRE	6-9
<i>Foeniculum vulgare</i>	fennel	GRE	7-9
<i>Oenanthe</i> sp.	parsley, dropwort	GRE	6-9
<i>Pastinaca sativa</i>	wild parsnip	GRE	6-9

Table 3.2. (a) Summary of pollen loads carried by females returning to the nest. **(b)** Summary of pollen ball composition. All percentages rounded to nearest 5; week is the week in the season and all samples were collected in 1992.

A.

Female	Week	% <i>B. napus</i> pollen	% other pollen types	Total number of pollen types
A	4	100	0	1
B	6	20	70, 10	3
C	6	10	50, 40	3
D	7	80	10, 10	3
E	11	0	50, 20, 20, 10	4
F	12	0	-	4
G	12	0	-	2
H	12	0	-	2

B.

Pollen Ball	Week	% <i>B. napus</i> pollen	% other pollen types	Total number of pollen types
A	7	50	15, 15, 10, 10	5
B	7	50	20, 20, 5, 5	5
C	7	60	20, 10, 10	4
D	7	30	30, 20, 15, 5	5
E	7	10	30, 25, 10, 10, 10, 5	7
F	9	55	15, 10, 10, 10	5
G	9	65	25, 10	3
H	9	0	70, 20, 10	3
I	9	55	20, 15, 10	4

Table 3.3. The parasites and parasitoids associated with halictids.

<u>Organism</u>	<u>Mode</u>	<u>Host</u>	<u>References</u>
Microorganisms:			
viruses	infect mature larvae	<i>Nomia</i> sp.	Stephen <i>et al.</i> 1969
bacteria <i>Bacillus apisepcticus</i>	infect mature larvae	various sp.	Batra 1965; Stephen <i>et al.</i> 1969
Fungi	pollen mass	<i>H. rubicundus</i>	Batra 1965; Batra & Bohart 1969; pers. obs.
protozoan (gregarine)	in hind gut of adult	<i>H. zephyrum</i>	Batra 1965
Nematodes: <i>Acrostichus</i> sp.	in haemolymph around ovaries	<i>Halictus</i> sp.	Batra 1965, Giblin <i>et al.</i> 1983
Strepsiptera: Stylopidae	infest egg and mature in adult	<i>Halictus</i> sp.	Batra 1965; Stephen <i>et al.</i> 1969; O'Toole 1991
Coleoptera: Meloidae	feed on pollen mass	various sp.	Batar 1965; Roubik 1989
Collembola:	scavengers in cell	<i>L. zephyrum</i>	Batra 1965
Diptera:	<i>Zodion cinereum</i>	<i>H. rubicundus</i>	Colyer & Hammond 1951; Else, in prep.
	other Conopidae	various sp.	Kerer & Atwood 1967
	Sarcophagidae sp.	<i>L. zephyrum</i>	Batra 1965
	<i>Leucophora grisella</i>	<i>H. rubicundus</i>	Batra 1965; Roberts 1973; This thesis
	<i>Bombylius</i> sp.	<i>Halictus</i> sp.	Batra 1965; Michener 1974; O'Toole 1991
	<i>Metopia</i> sp.	<i>Lasioglossum</i> sp.	Wcislo 1986
Hymenoptera: <i>Sphecodes</i> sp.	on pollen mass	<i>H. rubicundus</i>	Betts 1986; This thesis
		various sp.	Michener 1974; Roubik 1989; O'Toole 1991
	attack larvae	various sp.	Knerer & Atwood 1967; Roubik 1989
	attack larvae	various sp.	Michener 1974; Roubik 1989
Other arthropods: mites	scavengers in cell/ectoparasites	various sp.	Knerer & Atwood 1967, Stephen <i>et al.</i> 1969

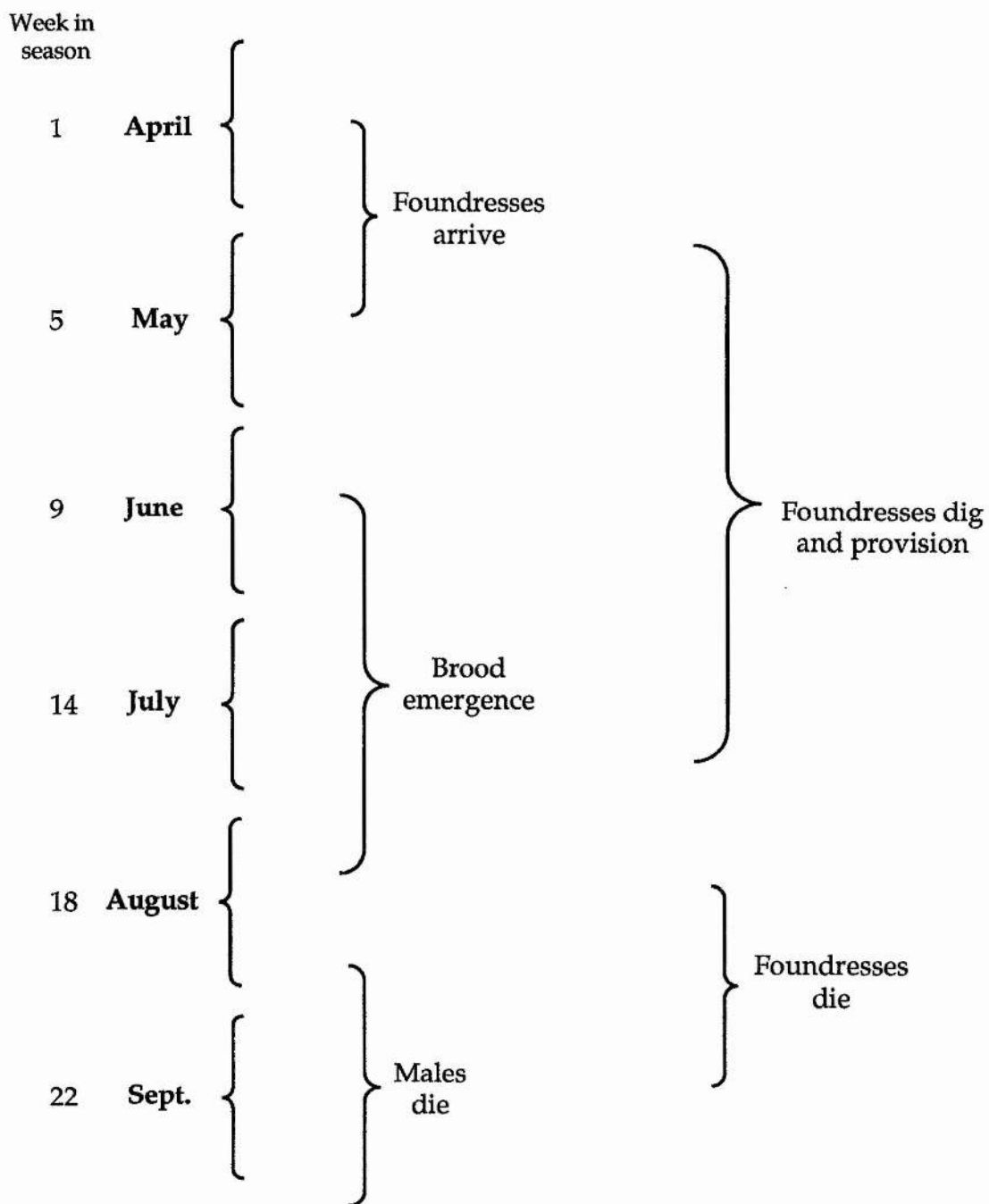


Fig 3.1. The phenology of *Halictus rubicundus* at Invergowrie.

A.



B.

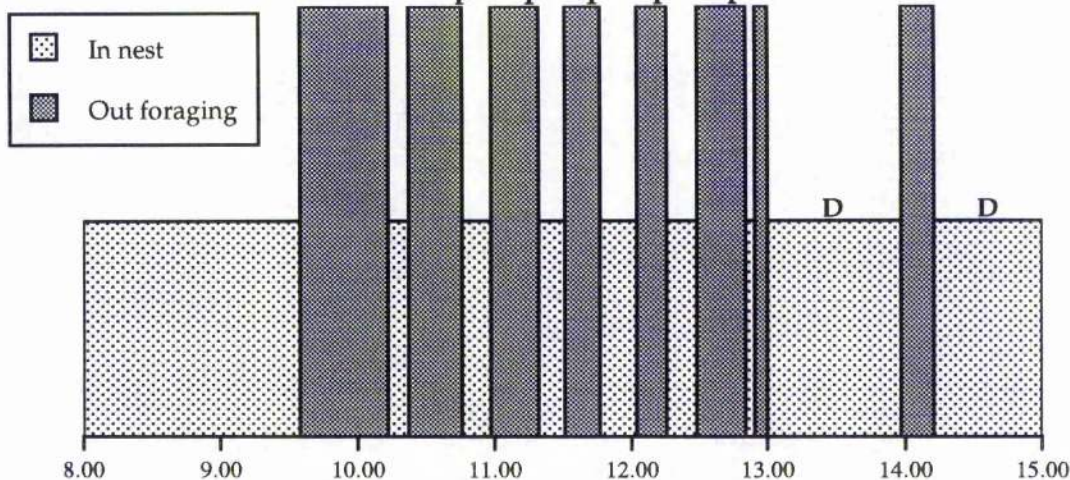


C.

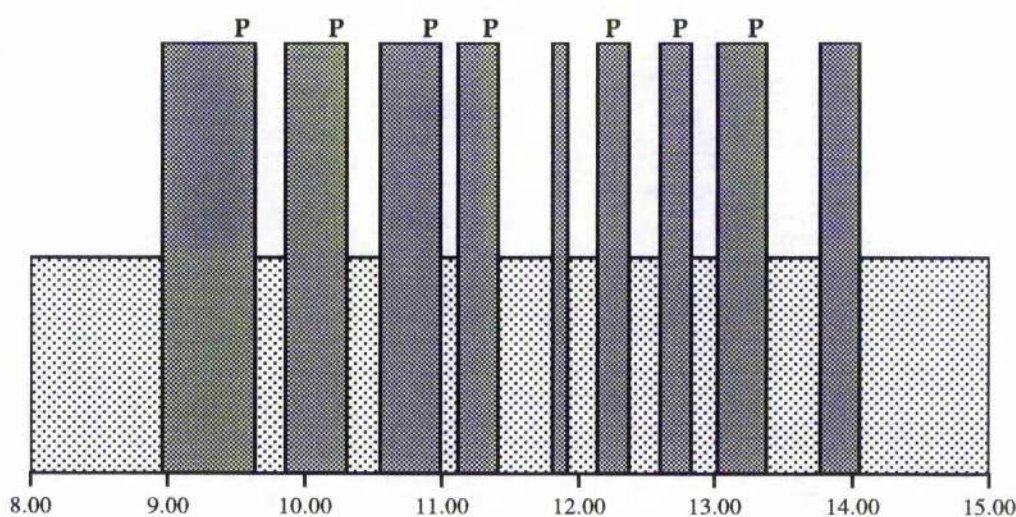


Fig. 3.2. Nest contents excavated from Kildale (summer 1994) (a) *H. rubicundus* larva and pollen ball; (b) *H. rubicundus* prepupae; (c) *H. rubicundus* pupa.

A.



B.



C.

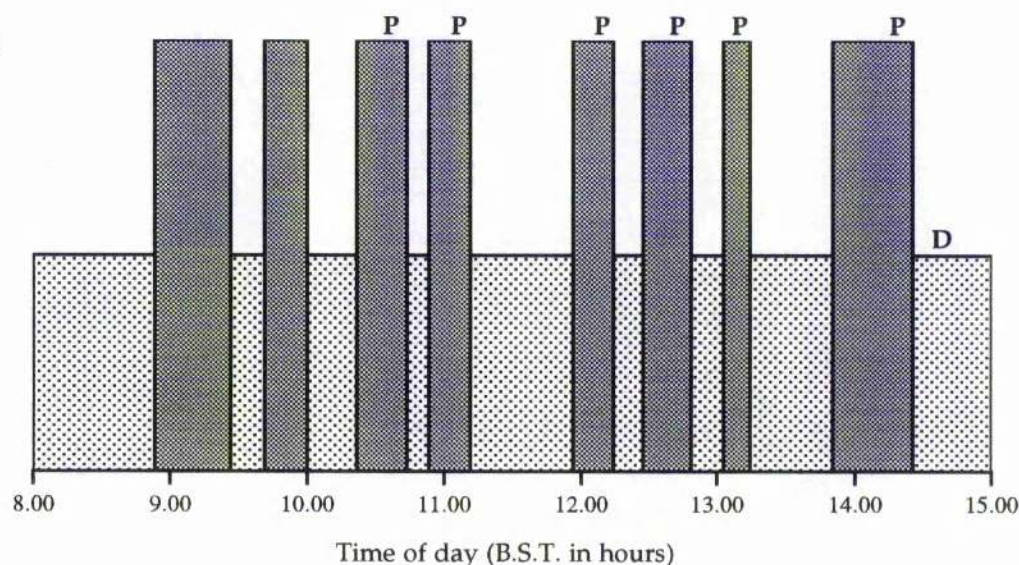
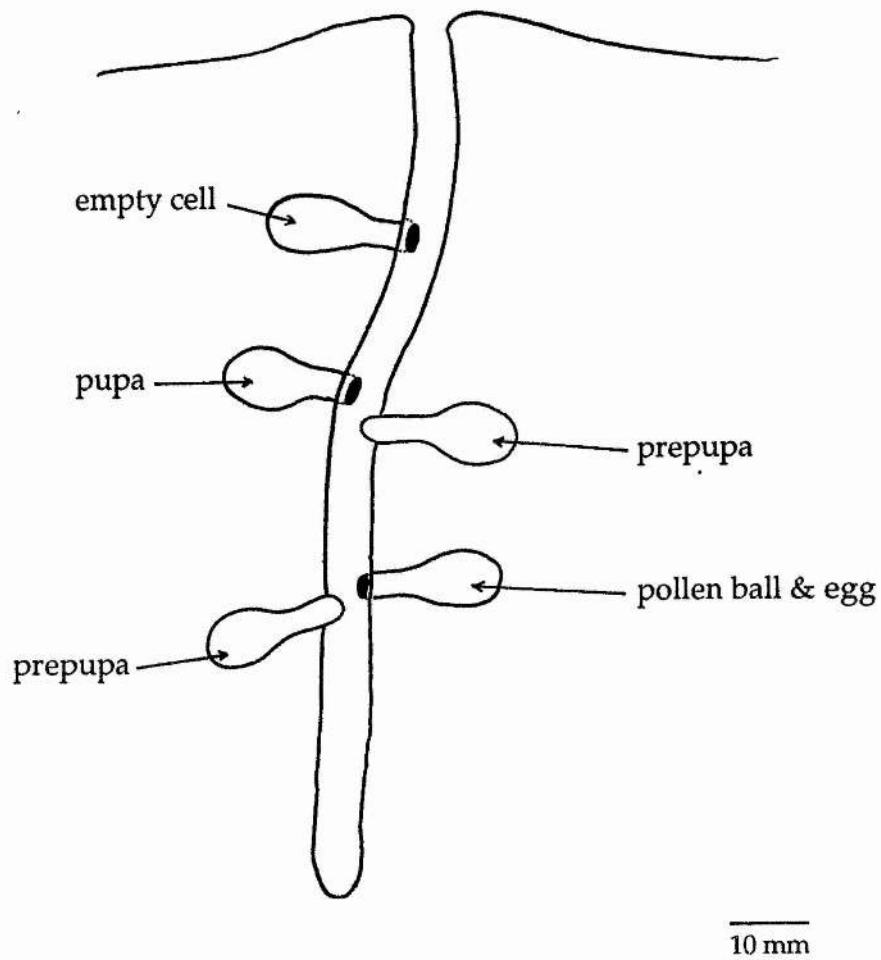


Fig 3.3. Daily activity cycle of females: (a) orange 13; (b) red 87; (c) orange 26. 'P' above bar indicates female returned to nest with pollen load; 'D' above bar indicates digging activity. Observations made at Invergowie on 10.6.94 & 13.6.94 (days when foraging was not limited by poor weather conditions).

A.



B.

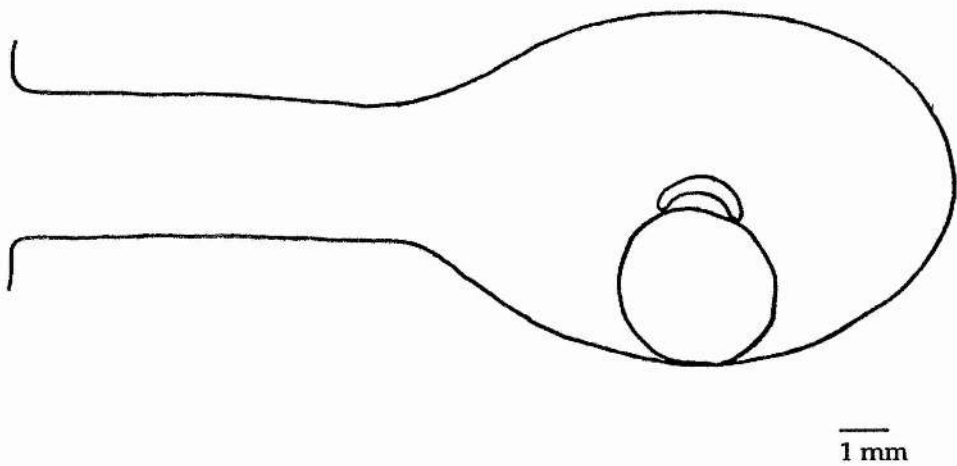
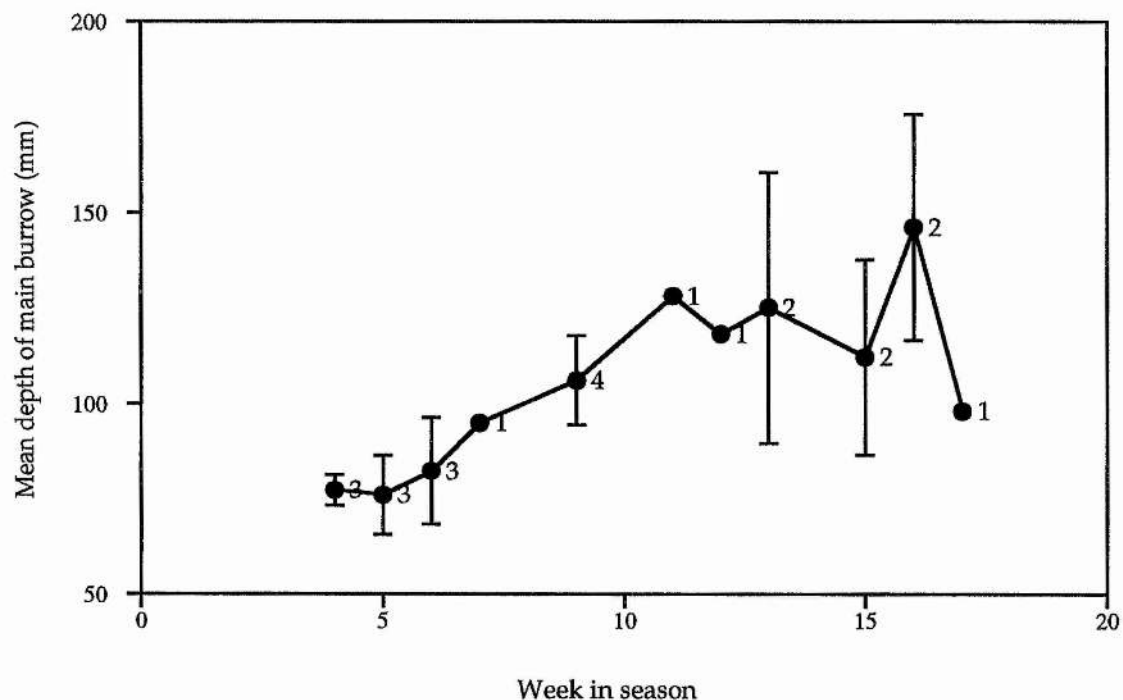


Fig. 3.4. (a) Diagrammatic representation of a mature nest excavated at Invergowrie on 5.7.94. (b) Diagrammatic representation of a typical brood cell with a pollen ball and egg.

A.



B.

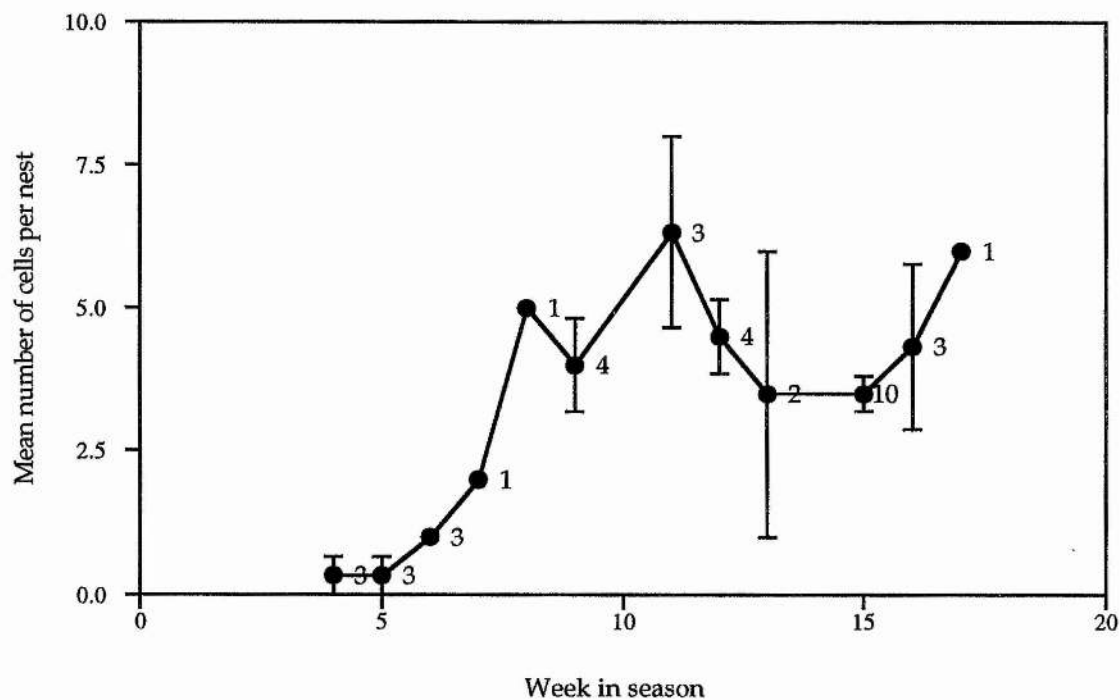
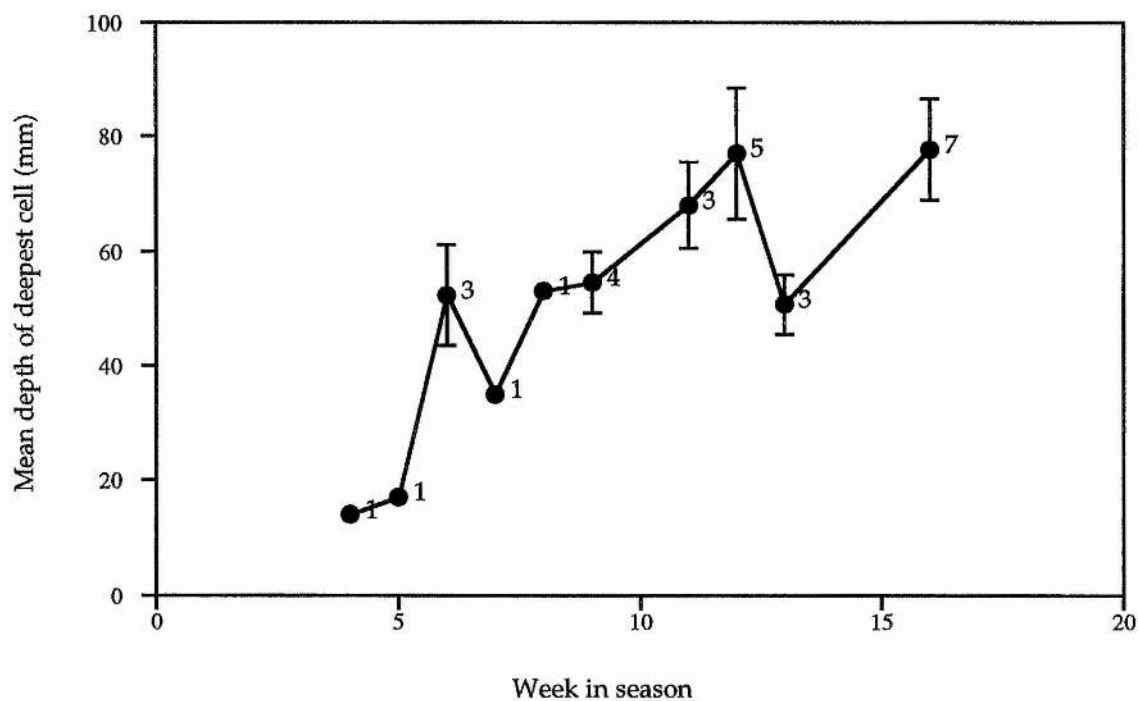


Fig 3.5. Results of nest excavations at Invergowrie. (a) Mean depth of main burrow versus week in season. (b) Mean number of cells per nest versus week in season).

A.



B.

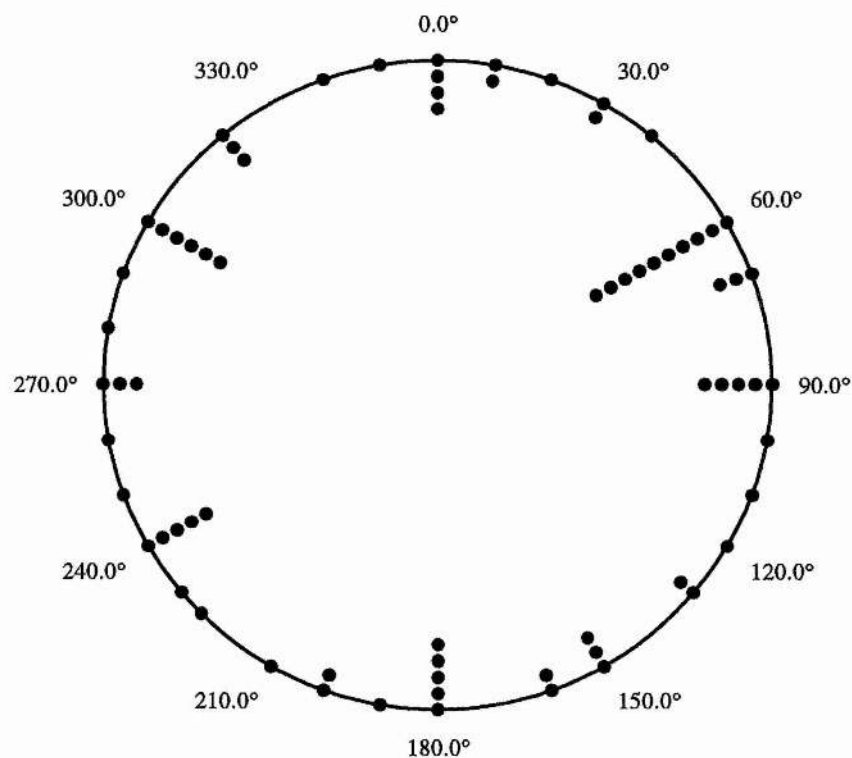


Fig 3.6. (a) Mean depth of deepest cell versus week in season. (b) Orientation of cells around main burrow; magnetic north is 0°; Rayleigh's test ($z = 0.6165$, $n = 78$, $p > 0.5$).

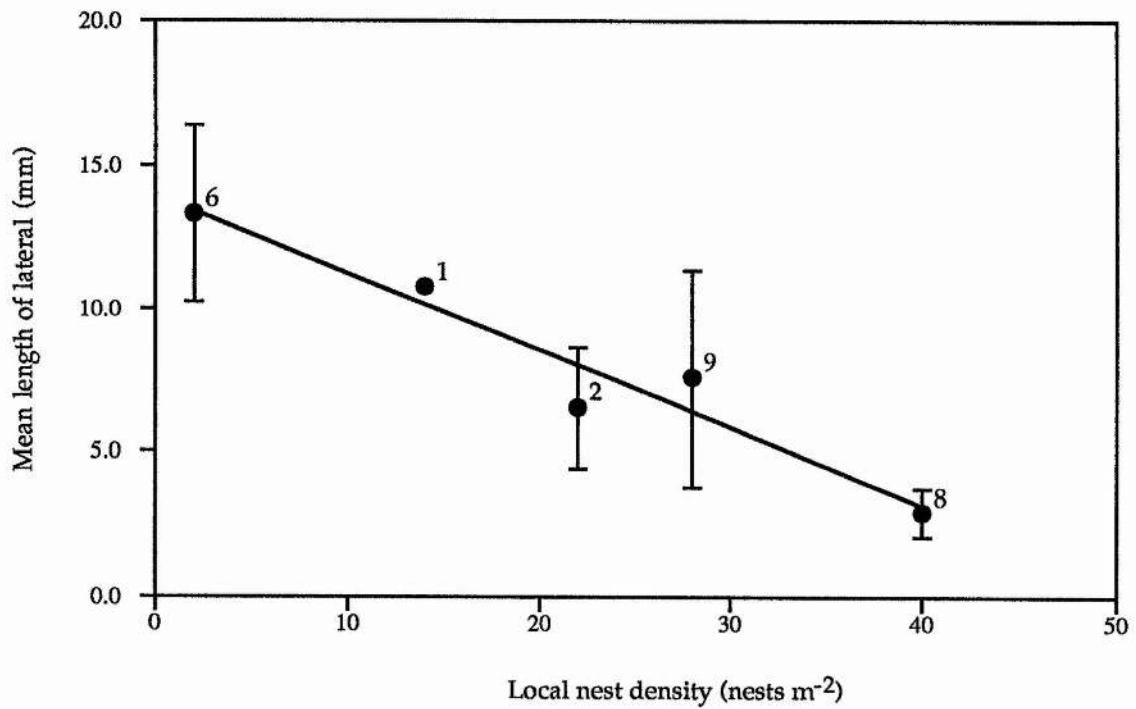


Fig 3.7. Lateral length and local nest density ($y = 13.9 - 0.271x$, $r^2 = 0.938$, d.f. = 3, $p = 0.007$).

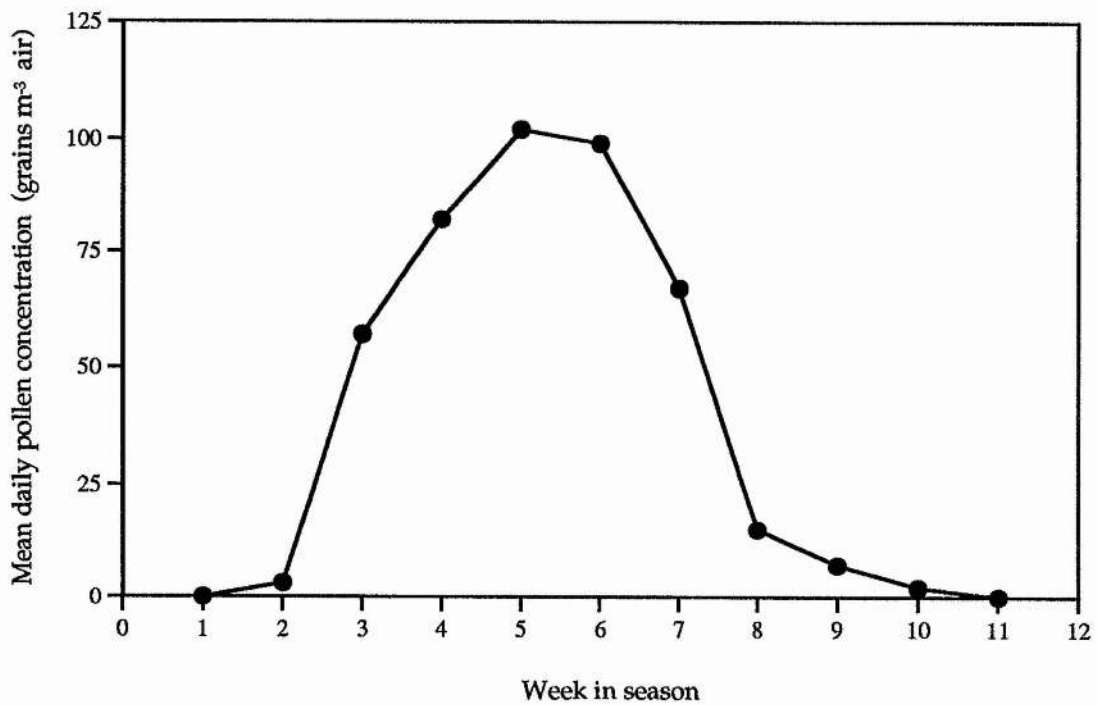
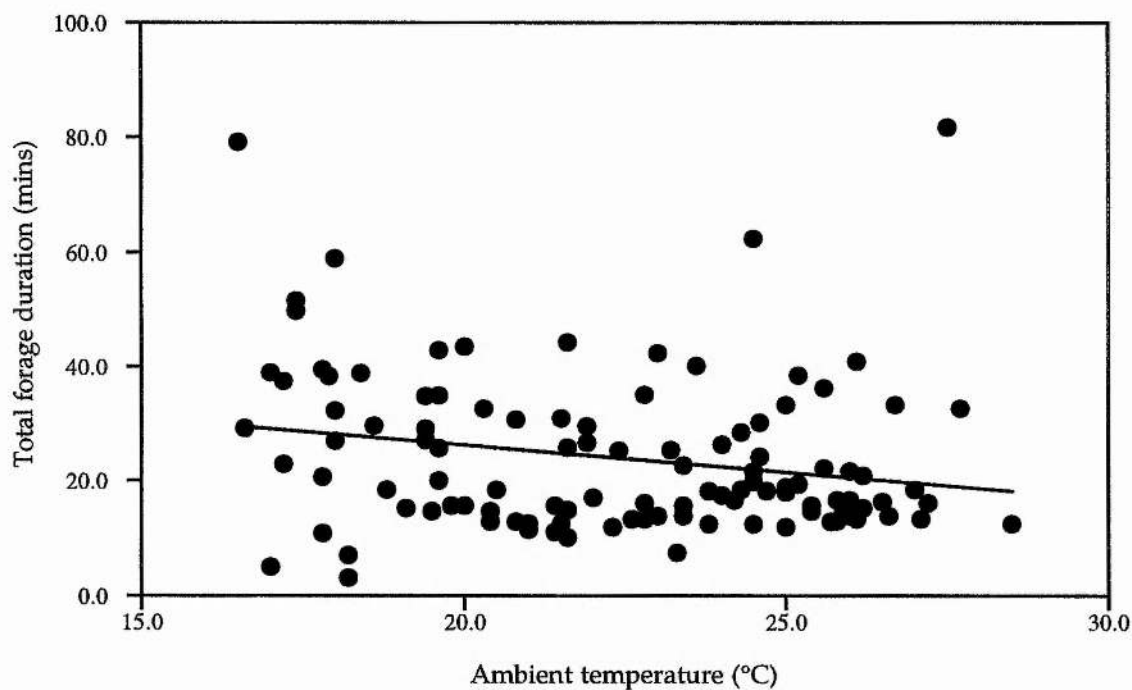


Fig 3.8. Mean daily pollen concentration of *B. napus* (averaged over each week). Mean of three years recordings (1992 - 1994) from Timmons *et al.* (in press).

A.



B.

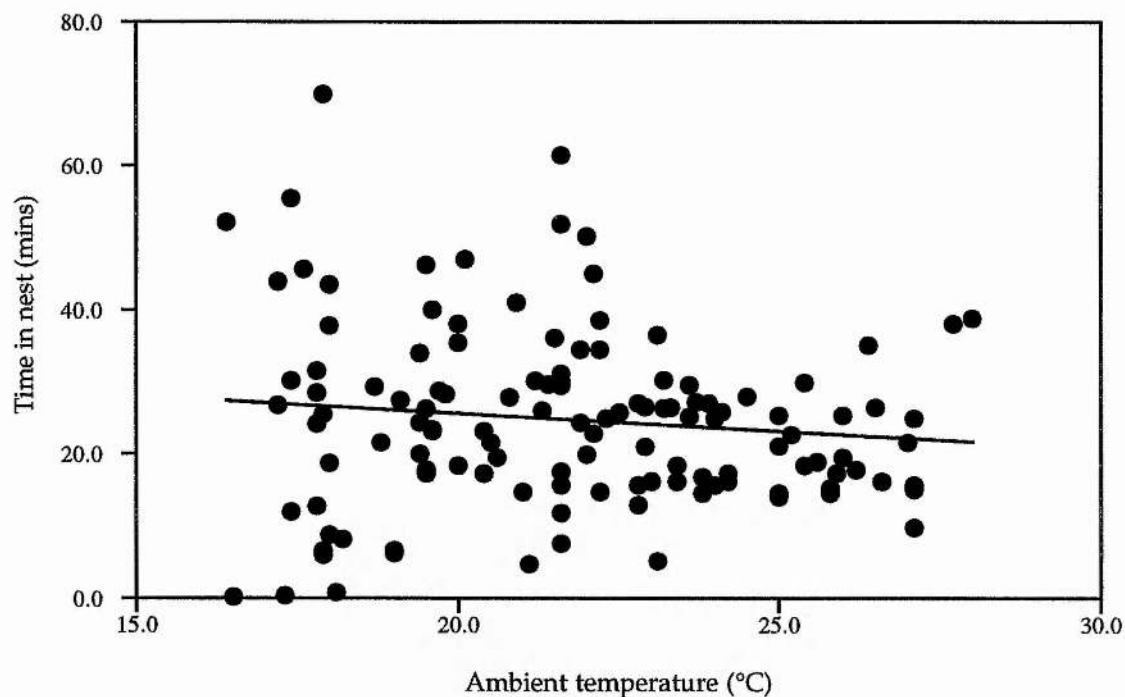
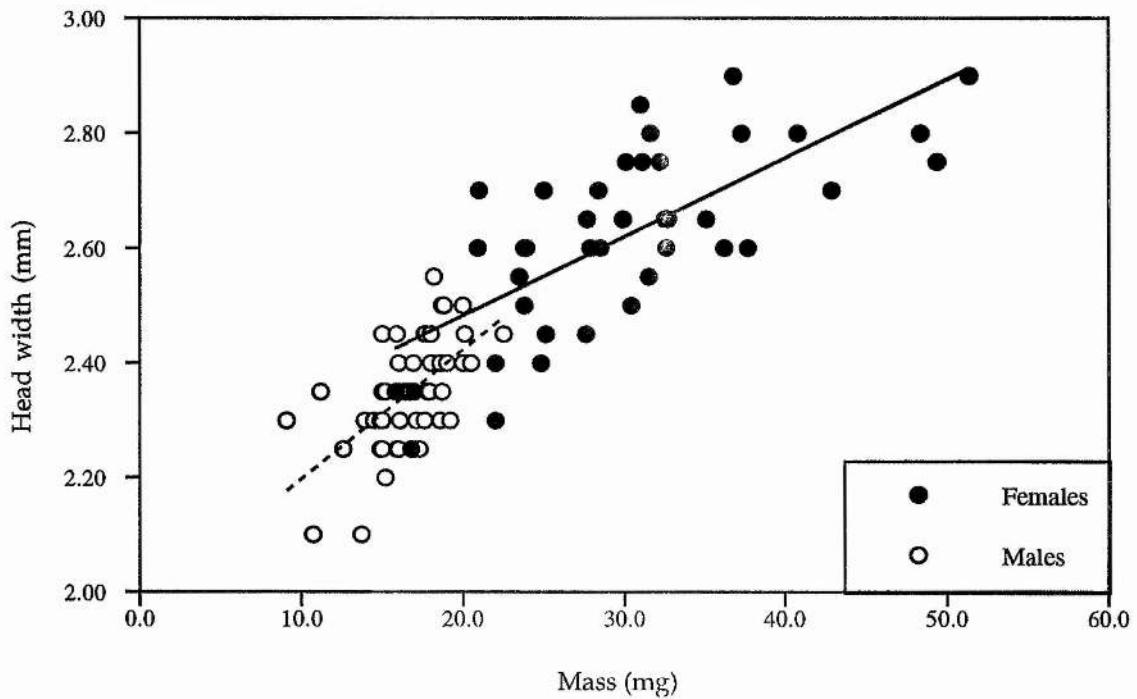


Fig 3.9. Foraging data collected on several days throughout the 1993 field season. **(a)** Duration of forage trip and ambient temperature ($y = -0.947x + 45.163$, $r^2 = 0.047$, d.f. = 108, $p = 0.023$). **(b)** Time spent in nest between foraging trips and ambient temperature ($y = -0.493x + 35.521$, $r^2 = 0.014$, d.f. = 122, $p = 0.196$).

A.



B.

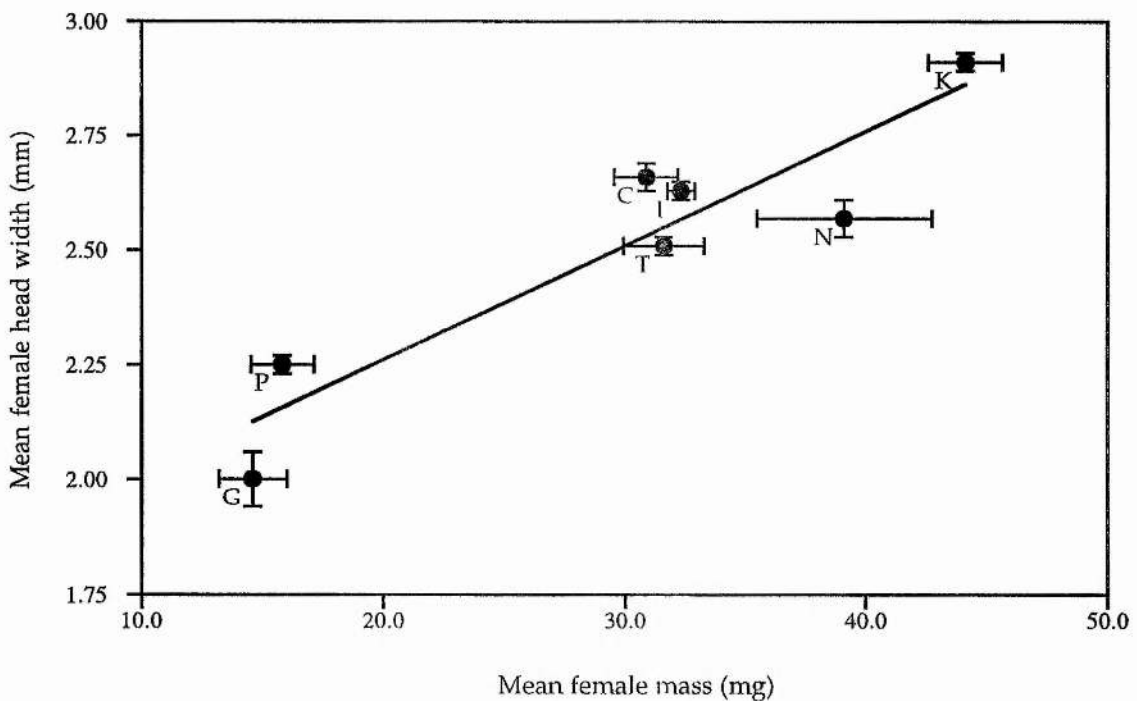
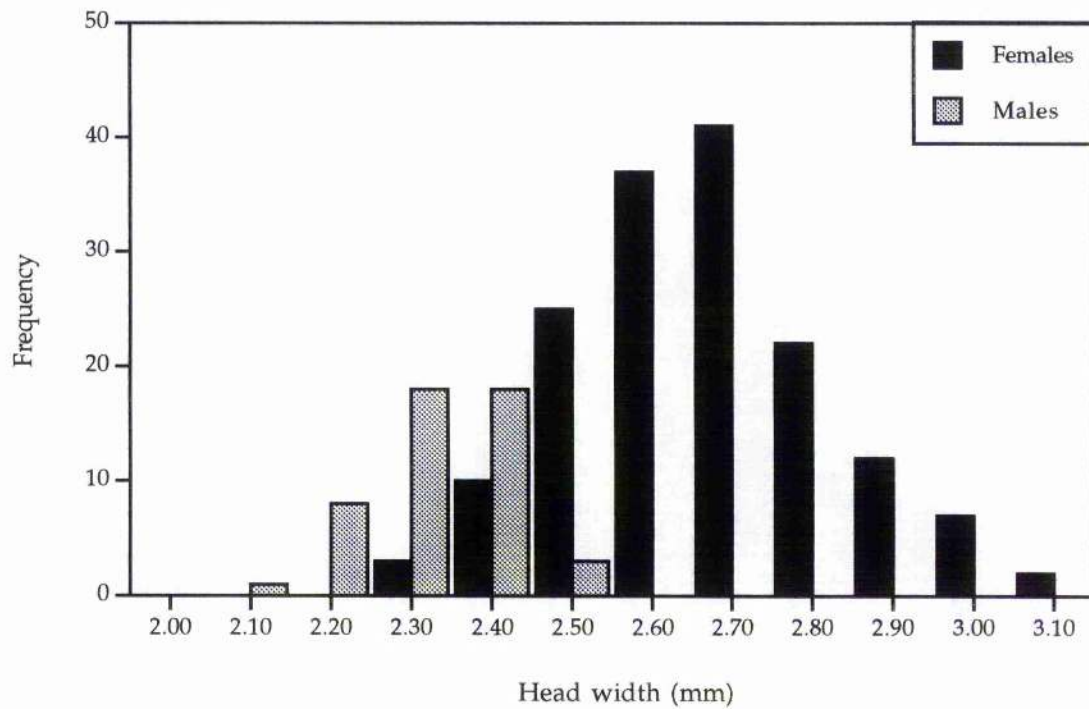


Fig 3.10. (a) Head width and body mass for females ($y = 0.014x + 2.209$, $r^2 = 0.500$, d.f. = 38, $p < 0.001$) and males ($y = 0.023x + 1.972$, $r^2 = 0.353$, d.f. = 46, $p < 0.001$) at Invergowrie. **(b)** Mean female head width and mean female body mass for all sites ($y = 0.025x + 1.763$, $r^2 = 0.854$, d.f. = 5, $p = 0.003$).

A.



B.

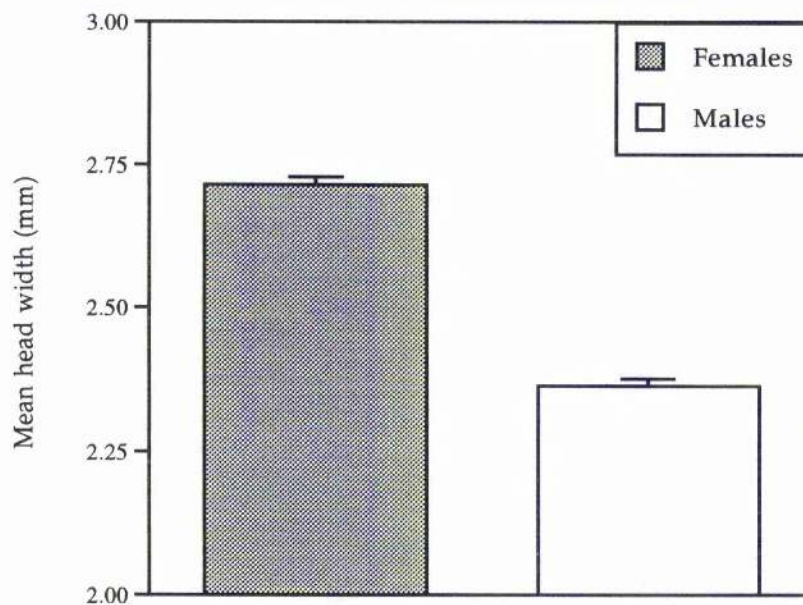
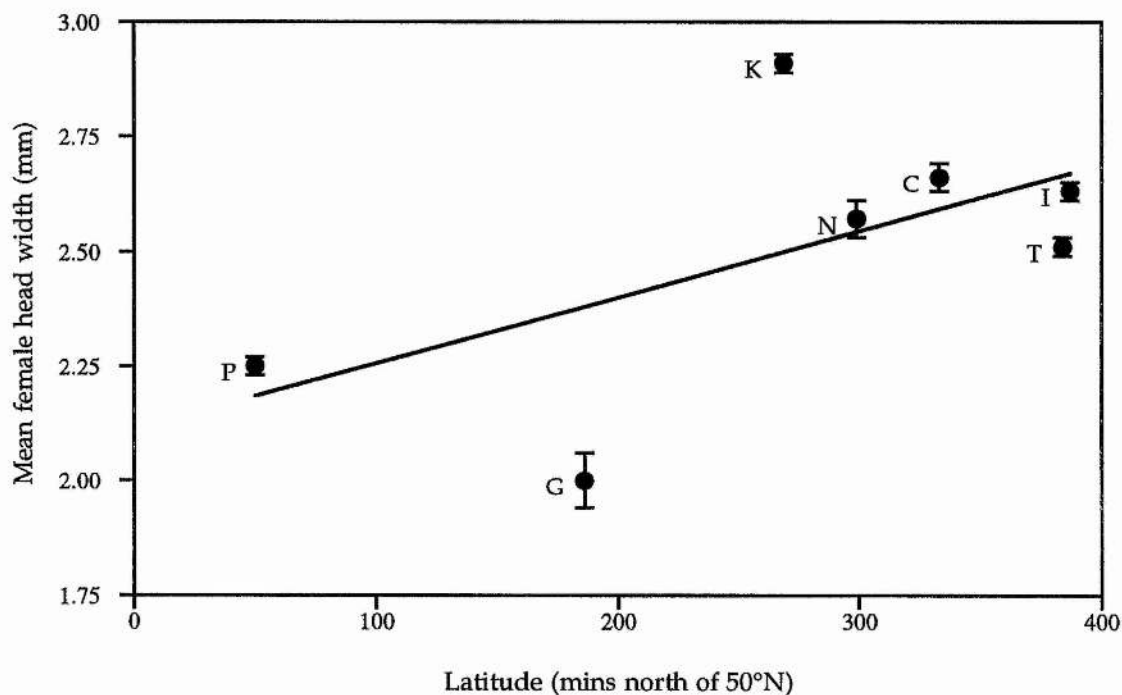


Fig 3.11. (a) Frequency distribution of male and female head widths at Invergowrie. (b) Mean female and male head widths (t test: $T = 19.07$, d.f. = 144, $p < 0.0001$).

A.



B.

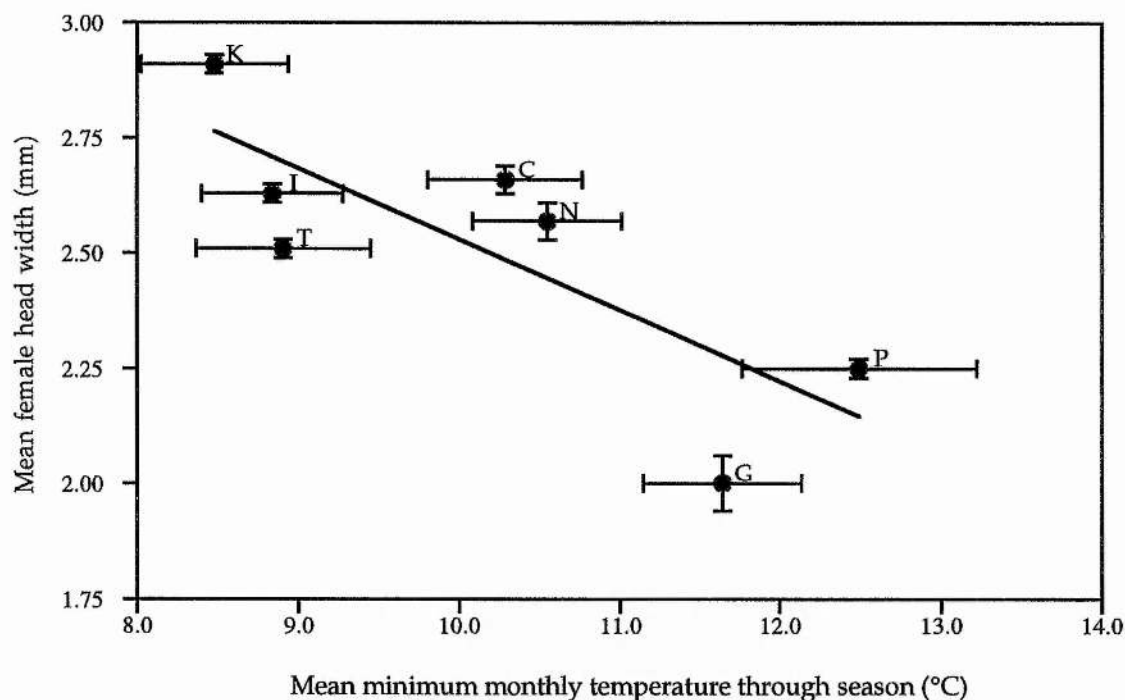


Fig 3.12. (a) Mean female head width and latitude ($y = 0.001x + 2.112$, $r^2 = 0.340$, d.f. = 5, $p = 0.169$). (b) Mean female head width and mean minimum monthly temperature through season ($y = -0.154x + 4.073$, $r^2 = 0.628$, d.f. = 5, $p = 0.036$).

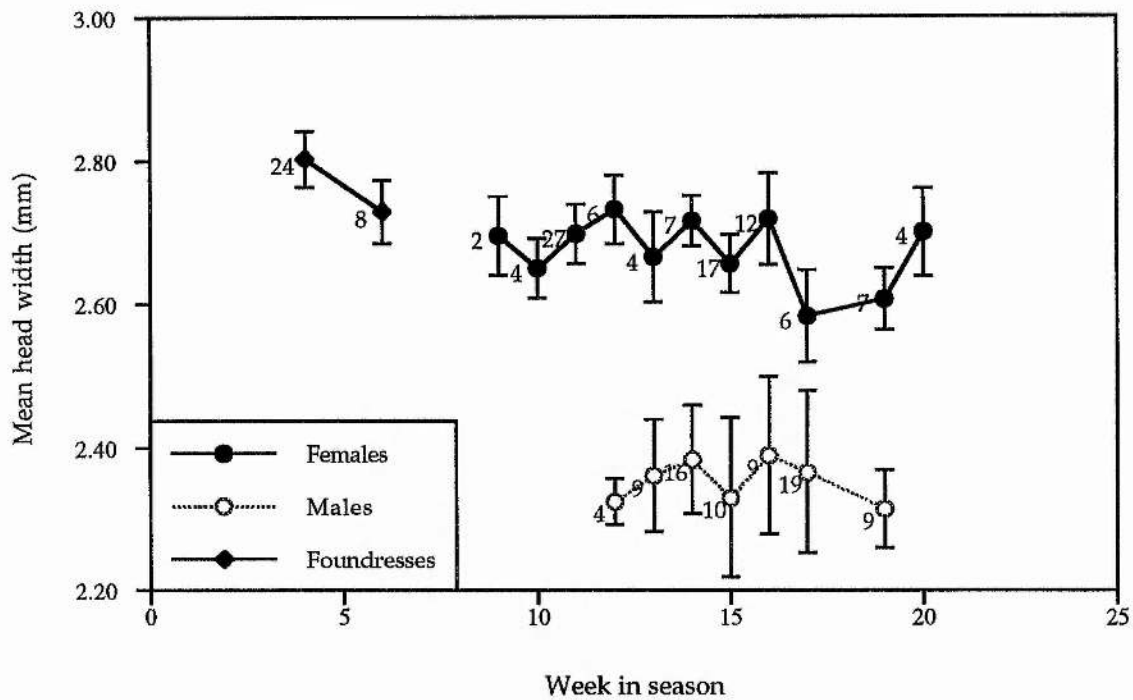
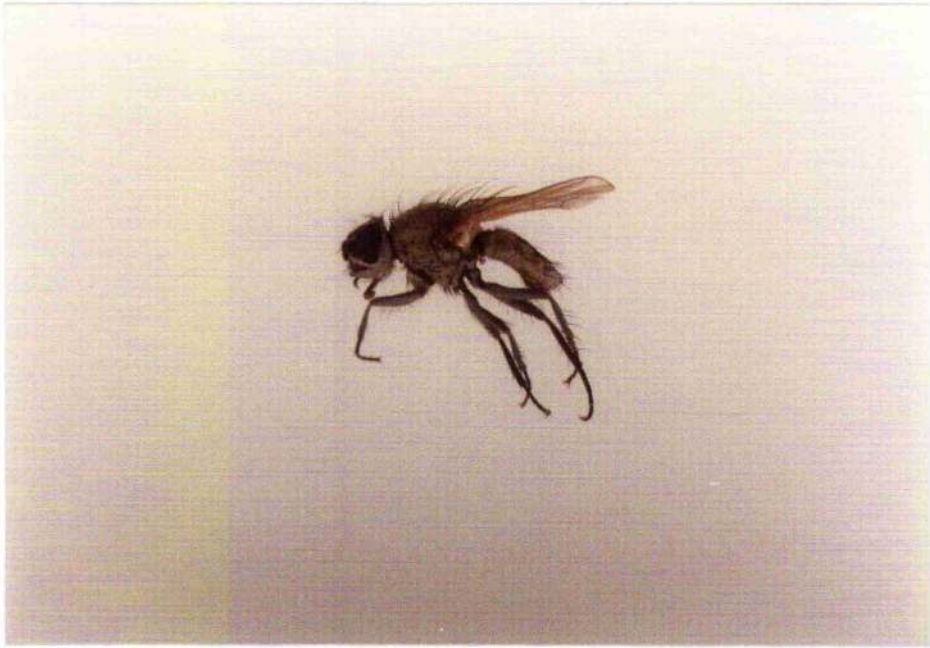


Fig 3.13. Mean weekly head width through season (grouped for 1992, 1993 & 1994) for foundresses, females (foundresses and newly emerged females) and males at Invergowrie.

A.



B.



Fig. 3.14. (a) Parasitic fly, *Leucophora grisella* (b) *L. grisella* oriented towards a *H. rubicundus* female as she enters her nest with a pollen load.

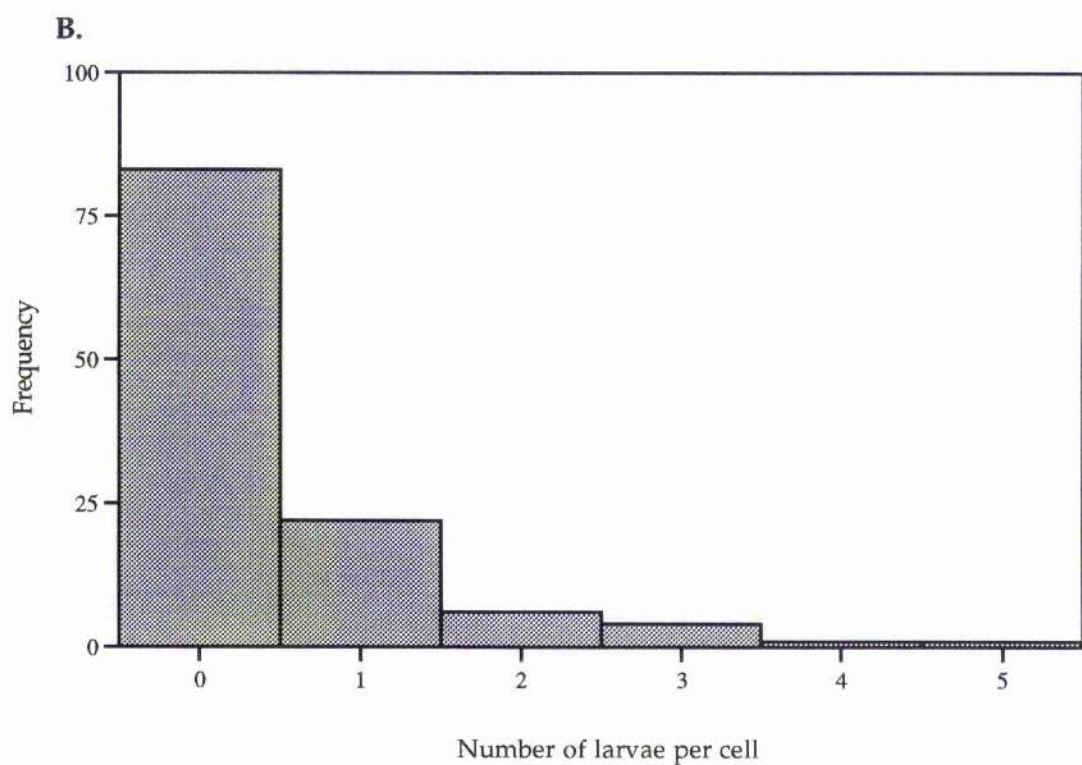
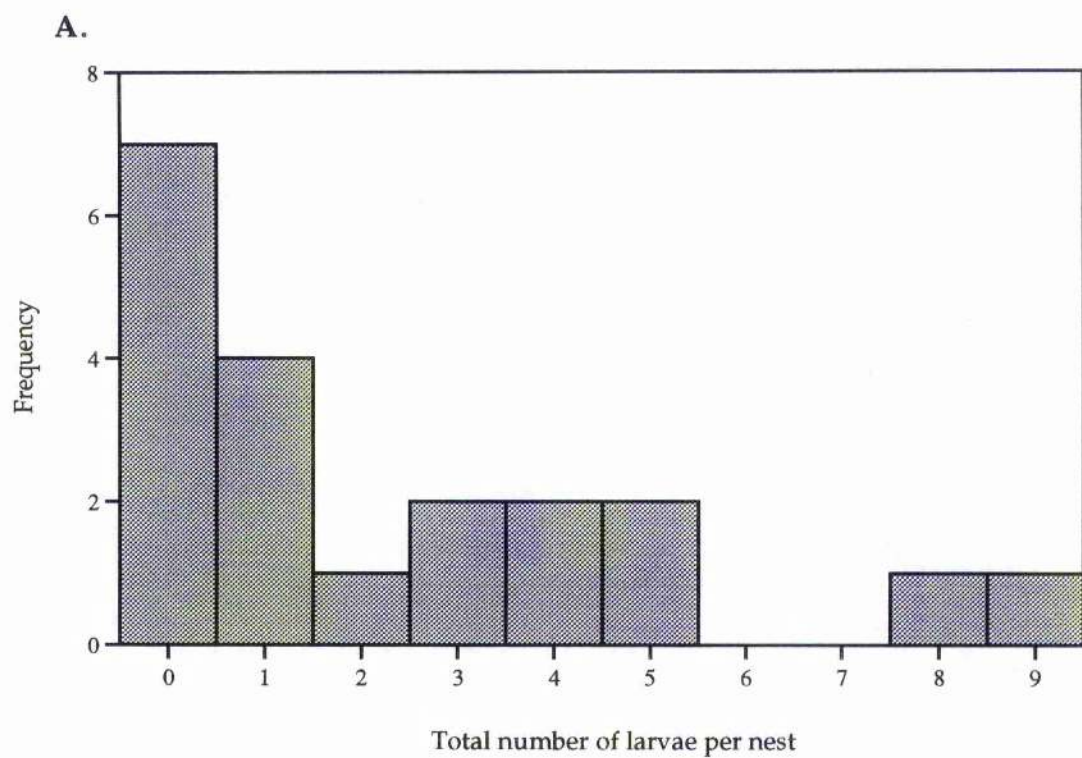


Fig. 3.15. Incidence of parasitic larvae in nests at Invergowrie. **(a)** Frequency distribution of total number of larvae per nest. **(b)** Frequency distribution for mean number of larvae per cell.

Chapter 4 Thermal biology

4.1 Introduction

4.1.1 Avenues of heat exchange

Insects exhibit relatively high rates of heat exchange with the environment as a consequence of their small size; their body temperatures are therefore intimately linked to those of their surroundings. Body temperature is one of the primary determinants of the rates of biochemical reactions, and so has important influences upon the type and frequency of behaviours exhibited. An understanding of heat exchange, and an ability to quantify it, allows many of the observed activity patterns to be explained and various adaptive strategies to be appraised. In addition particular mechanisms of heat transfer (both physiological and behavioural) can be evaluated to determine their relative importance in the overall thermal ecology of an insect.

The net thermal flux through an organism depends upon a great many individual rates of heat gain and heat loss for the various avenues of heat transfer. These rates will in turn depend upon an array of physical properties of the organism and its surrounding environment. Not surprisingly then, it is a complex matter to combine all the biophysical equations describing heat transfer and an exceedingly difficult procedure to measure them all individually. Extensive theoretical modelling has been done by Leyton (1975) and Stevenson (1985) such that, under specific conditions, many of the thermal balance equations can be applied and predictions of an organism's body temperature made. Bartlett and Gates (1967) completed a comprehensive analysis of the heat balance of a lizard, but few other studies have repeated the detail of this study. Other workers have overcome the need for the measurement of many of the more difficult environmental

parameters by using standard operative temperatures (also called black globe temperatures) which conveniently summarise, in a single measure, several effects of the thermal environment (see section 4.3.3 for full discussion).

There are four avenues of heat exchange between a body and its surroundings which are applicable to insects: radiation, convection, conduction and evaporation. The relative importance of these four modes will not only vary greatly with species but also with the physiological state of the individual and the specific microclimatic conditions in which it is situated. It is generally recognised, however, that the primary avenues of heat exchange for an insect, under most conditions, are radiation and convection (Willmer 1982; Casey 1988, 1992; but see also section 1.1) and these are represented in Figure 4.1. The rate of heat transfer will depend upon the physical properties of the insect; with the most important of these being size. In fact the mass, surface area, geometry and colour all influence the rates of heat gain and heat loss. Metabolic heat will also contribute to the body temperature of the insect; this may be an obligatory heat gain as a product of the level of activity being undertaken (such as resting or flying) or a voluntary strategy used to generate heat when required (basking or thermogenic shivering).

4.1.2 Physics associated with heat transfer

A. Radiation

The usual avenues of radiative heat exchange for a bee are shown in Figure 4.1, and it can be seen that the system is composed of several separate radiation components which combine to produce the net radiation flow. Any object (above absolute zero) will radiate electromagnetic energy according to its absolute temperature, and the rate of heat transfer is given by the equation:

$$Q_r = C_r \cdot A \cdot (T_s^4 - T_e^4) \quad [1]$$

where Q_r (W) is the rate of radiant heat loss or gain, A (m²) the area participating in the radiant heat transfer, T_s (°K) the surface temperature of the radiating object and T_e (°K) the mean temperature of the surroundings. The coefficient C_r incorporates the Stefan-Boltzmann constant as well as the emissivity of both the object's surface and of the solid surfaces making up the radiant environment (Louw 1993).

Solar radiation, both direct and reflected, is shortwave (SW) radiation with a peak wavelength of 0.5 μ m since it is emitted from the sun which is at ≈ 6000 °K (Wien's Displacement Law). In the UK the maximum radiation reaching the earth on a clear day mid summer is about 800 to 1000 W m⁻² (pers. obs.). Energy emitted from the sun is very constant, but the amount of SW radiation reaching an area of ground will vary greatly depending upon many factors; including season, time of day and aspect. The ground typically absorbs 90 % of the direct solar radiation ($\alpha = 0.9$, Leyton 1975), so the reflected radiation is reduced to approximately 10 % of the direct incoming value.

Longwave radiation (LW) from bodies at normal biological temperatures (0 - 50 °C) has a peak wavelength of about 10 μ m. SW solar radiation is absorbed by water vapour in the atmosphere and then re-radiated as LW radiation. Similarly the ground and the bee itself will absorb SW radiation and then re-radiate it as LW radiation; and this depends in part upon the emissivity (ϵ ; note that $\epsilon = 1 - \alpha$) of the surface of the body (see section 4.2).

For objects exposed to the direct sun on cloudless days the SW incoming radiation will be greater than the LW outgoing radiation and so they will warm up i.e. positive net radiation (Unwin & Corbet 1991). Conversely objects shaded from the

sun but still exposed to the sky will have a negative net radiation and so be cooler than air temperature.

The proportion of radiation received by an object depends upon its incidence to the direction of the incoming radiation. Lambert's Cosine Law states that the energy absorbed by a surface is proportional to the cosine of the angle between the direction of incident radiation and a line normal to the surface. This has important consequences for basking posture adopted by insects while basking, as the amount of solar radiation absorbed will vary greatly with the positioning of the individuals body relative to the sun. For radiation emitted from a surface this proportion also holds true (Kirchoff's Law).

Most living tissue virtually acts as a black body in the infrared region of the spectrum, and since nearly 50% solar energy is in the visible region of the spectrum it follows that colour could potentially have a large effect upon the quantity of radiant energy absorbed (Digby 1955). Colour affects the absorption and reflectance of SW radiation but not LW radiation. The reflectance of most insects fall between 2 and 20 % with most bees having reflectances of 4 - 10 % (Willmer & Unwin 1981).

B. Convection

The primary avenue of heat loss for bees is convection (see Appendix 1) and this is governed by the equation:

$$Q_c = C_c \cdot A \cdot V^n \cdot (T_s - T_a) \quad [2]$$

where Q_c is the rate of convective heat loss (W), A (m^2) the area of the animal taking part in the convective heat exchange, V ($m\ s^{-1}$) the wind speed, T_s ($^{\circ}C$) the

surface temperature of the animal and T_a (°C) the ambient temperature. The exponent n depends upon the size of the animal and also upon the range of fluid speeds over which the equation is applied. C_c varies not only with animal size but also with physical properties of the fluid including its density (Louw 1993).

When a layer of air becomes warmed by a surface it is in contact with, it becomes less dense and so rises thus removing thermal energy from the object. At low wind speeds (0 - 30 cm s⁻¹) this sort of free convection predominates and convective heat transfer is almost independent of velocity. At high wind speeds forced convection takes place as the warmed air is continually removed, thus maintaining the thermal gradient, and heat transfer then varies with the square root of the velocity i.e. $n = 0.5$ (Casey 1992). With higher velocities turbulence becomes an important factor which complicates the relationship. Heinrich and Buchmann (1986) demonstrated that cooling constants (see section 4.2.2) of dead specimens of *Xylocopa varipuncta* increase markedly with wind speed; the cooling rate of the thorax approximately doubled as air speed increased from 1 to 6.5 m s⁻¹. Furthermore the thoracic temperature of a bee undergoing simulated basking in still air was ≈ 40 °C which was significantly higher than the same bee in air moving at 6.5 m s⁻¹ whose thoracic temperature was ≈ 35 °C.

C. Conduction and evaporation

Conduction is the movement of heat by interaction of adjacent molecules without the mass motion of the medium through which the energy takes place. The rate of conduction is expressed by the equation:

$$Q_k = C_k \cdot A \cdot (T_1 - T_2) / L \quad [3]$$

where Q_k is the rate of heat transfer (W) by conduction through a segment of material of thickness L (m) and area A (m²), T_1 (°C) and T_2 (°C) are the face temperatures. Since the rate of conductive heat transfer is proportional to the area of contact, it is of very little importance for a bee walking or basking on a substrate as only a small area of the tarsi will be available for heat transfer. The one general exception will be when the bee is basking in its nest entrance before emerging. Then a substantial proportion of the thorax and/or abdomen may be pressed against the tunnel sides which during solar insolation will have an elevated temperature relative to that of the air (see section 4.3.2).

Evaporation can bring about cooling through the latent heat removed as water changes state. Although evaporative heat loss has been implicated in a few specialised mechanisms for the control of body temperature (Heinrich 1980a, b), under most circumstances the quantities of heat lost by evaporation are an order of magnitude or more lower than those lost by convection (Bartholomew 1981).

D. Mass, surface area and geometry

The most important size parameter affecting heat exchange of an insect with its surroundings is surface area. From the allometric relationship between the relative surface area and body mass (or volume); surface area is proportional to the two-thirds power of the volume. Hence the smaller the animal the greater the relative surface area and, all other factors being equal, the faster it will gain or lose heat (Casey 1981). The equations (1 to 3) describing radiative, convective and conductive heat exchange all have rates that are proportional to area (A).

A consequence of the higher rates of heat exchange in smaller insects of a given morphotype is that they will reach equilibrium body temperature more rapidly than larger ones under the same conditions. For ectotherms absorbing solar

radiation, heat transfer theory predicts that at thermal equilibrium a large insect will have a larger temperature excess (T_{ex}) than a smaller insect, owing to its greater heat capacity (Stevenson 1985). Larger individuals will also be less subject to transient changes in body temperature as a result of higher thermal inertia.

Body shape also influences the rate of heat exchange, thermal equilibrium and T_{ex} of an insect. For a given mass an animal having a body with more elongate elements and a filament-like geometry minimises radiant heat gain and maximises convective heat loss when exposed to insolation.

The amount of metabolic heat generated by a bee will be proportional to its mass (usually more directly to its thoracic mass); this is true whether the insect is resting, flying or actively warming as a prelude to some activity. Thus, under constant conditions, a larger individual maintains a higher T_{ex} as the smaller individual loses a disproportionately greater amount of heat due to its higher surface area to volume ratio.

4.2 Laboratory investigation of heat exchange

4.2.1 Surface area and reflectance

Three individual *H. rubicundus* with masses equal to (or very close to) the mean of each sex were selected and their surface areas determined. For females this was found to be $80.1 \pm 5.6 \text{ mm}^2$ (3) and for males $57.6 \pm 3.7 \text{ mm}^2$ (3). Using the mean masses for these same individuals ($30.5 \pm 0.2 \text{ mg}$ (3) and $16.9 \pm 0.2 \text{ mg}$ (3) respectively) gave surface area to mass ratios of $2.6 \text{ mm}^2 \text{ mg}^{-1}$ for females and $3.5 \text{ mm}^2 \text{ mg}^{-1}$ for males. Thus for these mean individuals, males have approximately 1.35 as much surface area per unit mass as females do.

At the extremes of size, the male and female bees just overlap, and the surface area of a 22.5 mg male and a 22.0 mg female were calculated. The male had a surface area to mass ratio of $2.86 \text{ mm}^2 \text{ mg}^{-1}$ and the female a ratio of $2.97 \text{ mm}^2 \text{ mg}^{-1}$. It therefore appears then for a given mass males and females have the same surface area to volume ratio. In later analyses these same two bees will be referred to as the large male (male 3) and small female (female 1).

The mean reflectance of females was found to be $7.6 \pm 0.2 \%$ (30) and for males $6.7 \pm 0.2 \%$ (30). A two-tailed t test showed that females were significantly more reflective than males ($T = -2.74$, d.f. = 56, $p = 0.0082$). Both sexes fall in the middle of the range of reflectances shown by other Hymenoptera as measured by Willmer and Unwin (1981). Males and females had little pubescence on the thorax relative to many other bee species, and there was no apparent difference between sexes (pers. obs.).

Males, being smaller, consequently have a higher surface area to volume ratio and this in addition to their lower relative reflectance predicts that heat exchange rates should be greater in males than in females.

4.2.2 Cooling constants and conductance

The way a body cools under constant environmental conditions is described by Newton's Law of cooling. This states that the rate of cooling of an inert body is proportional to the difference in temperature between the centre of the body and its surrounding medium. This relationship is expressed algebraically as:

$$\Delta T_{\text{th}}/\Delta t = k \cdot (T_{\text{th}} - T_{\text{a}}) \quad [4]$$

where T_{th} ($^{\circ}\text{C}$) is thoracic temperature, T_a ($^{\circ}\text{C}$) is ambient temperature, t (min) is time and k ($^{\circ}\text{C min}^{-1} ^{\circ}\text{C}^{-1}$) represents the cooling constant of a particular body. The cooling constant can be determined empirically by measuring the cooling curve of an animal in the laboratory, and plotting rate of cooling against T_{ex} ($= T_{th} - T_a$). The gradient of this line is then an estimate of k .

Multiplying the cooling constant by specific heat yields thermal conductance (C), the rate of heat flow from the core of the body to the environment. The specific heat capacity of insect tissue is taken to be $0.83 \text{ cal g}^{-1} ^{\circ}\text{C}^{-1}$ or $3.47 \text{ J g}^{-1} ^{\circ}\text{C}^{-1}$ (Heinrich 1975). The conductance defines the heat transfer from the core of the body to the environment, rather than from its surface to the environment as the cooling constant does.

The Newtonian model oversimplifies the situation since in the natural environment neither C nor T_{ex} are well defined (Casey 1988). Conductance will vary with microclimatic parameters such as wind velocity (Heinrich & Buchmann 1986) and the surrounding T_a is continually modified by the temperature of the animal. However, since radiative and convective heat exchange are both approximately linearly related to temperature differential, this model is justifiable in its application under certain circumstances (Casey 1992).

The cooling constants calculated for chilled bees warming to T_a while dead or alive are given in Table 4.1. None of the bees studied exhibited a measurable T_{th} over the surrounding T_a , thus indicating a lack of endothermic warming under these conditions. Analysis of the individual paired rates for live and freshly killed bees revealed no significant difference in the rates under each condition ($T = -1.22$, $n = 6$, $p = 0.28$). This could be further evidence to support the case for the absence of endothermic warming in *H. rubicundus*. It may, however, be that warming from

10 °C to T_a passively is favoured by the bee as actively warming-up is energetically expensive and probably unnecessary.

All the cooling constants were slightly greater for the live bee than for the dead bee (except for Male 1), and this is most likely due to the small amount of heat from basal metabolic processes produced during the passive warming (Figure 4.2). Such additional heat would marginally increase the rate of warming and thus inflate the cooling constant slightly. An alternative explanation is that the lower warming rate of dead bees is due to a decrease in the functional surface area to volume ratio of the thorax as a result of the bees curling their legs up tightly underneath the thorax when they die.

For *H. rubicundus*, after grouping the live and dead values of k there was a significant difference between the sexes (oneway ANOVA: $F = 15.14$, d.f. = 11, $p = 0.003$), with males having larger cooling constants than females. Within each sex there was also an effect of size (for females, oneway ANOVA: $F = 10.90$, d.f. = 5, $p = 0.042$ and for males, oneway ANOVA: $F = 8.62$, d.f. = 5, $p = 0.057$). In both cases cooling constants and body size were inversely related (value of k for three sizes: small > medium > large). Data from more individuals would further quantify this relationship. The values for the largest male and smallest female are almost identical; this is not surprising as they share the same surface area to volume ratios.

The relationship between cooling constants and size agrees with those from other studies (May 1976a, Bartholomew 1981, Stone 1993). Assuming that k is the same for the thorax as for the whole bee, the thoracic masses of Female 2 (body mass = 30.1 mg, thoracic mass = 9.1 mg) and Male 2 (body mass = 16.1 mg and thoracic mass = 4.9 mg) can be used to predict the cooling constant from the equation given by May (1976a, Figure 7) for live bees:

$$\log k = -0.451 \cdot \log \text{thoracic mass} - 0.848$$

This equation used data for thoracic masses greater than 30 mg and so can be examined to see if the relationship approximately holds at much lower masses. May's prediction for the female is $1.18 \text{ }^{\circ}\text{C min}^{-1} \text{ }^{\circ}\text{C}^{-1}$ and the measured value for *H. rubicundus* is $1.30 \text{ }^{\circ}\text{C min}^{-1} \text{ }^{\circ}\text{C}^{-1}$, and the same values for the male are $1.56 \text{ }^{\circ}\text{C min}^{-1} \text{ }^{\circ}\text{C}^{-1}$ predicted and $1.64 \text{ }^{\circ}\text{C min}^{-1} \text{ }^{\circ}\text{C}^{-1}$ measured. There is then a good agreement between May's predictions and the results obtained for *H. rubicundus*.

The mean conductance values for live females and live males, calculated from Table 4.1, are $0.076 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ ($1.085 \text{ cal min}^{-1} \text{ g}^{-1} \text{ }^{\circ}\text{C}^{-1}$) and $0.098 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ ($1.404 \text{ cal min}^{-1} \text{ g}^{-1} \text{ }^{\circ}\text{C}^{-1}$) respectively. These values are considerably larger than the values obtained for other Hymenoptera, but are easily explained by the small size and lack of pubescence in *H. rubicundus*. For instance the large and highly pubescent bee *Anthophora plumipes* has a conductance of $0.03 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ (Stone 1993); *Xylocopa varipuncta* has a conductance of $0.023 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ (Heinrich & Buchmann 1986), *Centris pallida* a conductance of $0.034 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ (Chappell 1984) and some euglossine bees have conductances of $0.026 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$. Two solitary bees of comparable size have conductances very similar to *H. rubicundus*: *Osmia leaiana* (52 mg), $0.077 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ and *Colletes daviesanus* (36 mg) $0.090 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ (Stone 1989).

4.2.3 Warming and cooling rates and temperature excesses

A series of initial warming and initial cooling rates were generated by warming dead bees with simulated solar radiation then allowing them to cool passively. As the previous section showed there was no significant difference in the cooling constants for live and dead bees. Dried dead bees were used to prevent the results

from being confounded by the loss of moisture from the samples over the course of the experiment, as would be the case with freshly killed bees. Wet mass and dry mass are highly correlated (females: $y = -6.80 + 0.640x$, $r^2 = 0.777$, d.f. = 15, $p = 0.004$ and males: $y = -0.556 + 0.456x$, $r^2 = 0.928$, d.f. = 14, $p < 0.001$), so this is a reasonable procedure to follow. Dried masses are obviously less than the live masses and so the associated rates of heat exchange will be higher; however, the form of the relationship between individuals will remain unchanged. Further the use of dried masses prevents the direct extrapolation of results to field data.

The initial warming rates of females of three different masses (Figure 4.3a) were, as expected, independent of final T_{ex} ; the regressions of initial warming rates on T_{ex} all have gradients that are not significantly different from zero (all r^2 values < 0.112 and p values > 0.321). The mean warming rates for the different size categories (table 4.2) were however significantly different (oneway ANOVA: $F = 77.46$, d.f. = 89, $p < 0.001$).

To allow direct comparison of the initial cooling rates the three values at $\approx 5^\circ\text{C}$ have been treated separately from the rest of the curve. The initial cooling rates of the three females increased with their temperature excess, as Newton's law of cooling would predict (Figure 4.3b). The mean cooling rates at a T_{ex} of 5°C (Table 4.2) differed significantly from each other (oneway ANOVA: $F = 21.61$, d.f. = 8, $p = 0.002$).

The initial cooling rates of males (Figure 4.4a) are also independent of T_{ex} (for all three regressions all r^2 values < 0.017 and p values > 0.498); and they are also significantly different from one another (oneway ANOVA: $F = 66.30$, d.f. = 89, $p < 0.001$). The initial cooling rate curves (Figure 4.4b) are indicative of a Newtonian cooling process and the rates at 5°C are different but not quite significantly so (oneway ANOVA: $F = 4.69$, d.f. = 8, $p = 0.059$).

If a male and female of the same size are compared (Figure 4.5) then there is no significant difference in the initial warming rates (oneway ANOVA: $F = 2.01$, d.f. = 59, $p = 0.162$) or the initial cooling rates (oneway ANOVA: $F = 1.98$, d.f. = 5, $p = 0.232$).

All the warming rates are substantially greater than the cooling rates, so that under 900 W m^{-2} of solar radiation the bees will quickly warm as passive heat loss is relatively small (this assumes that there is no forced convection taking place). From an overall comparison of the warming and cooling rates for both sexes the following relationship is apparent:

$$\text{♂}_{\text{small}} > \text{♂}_{\text{medium}} > \text{♂}_{\text{large}} = \text{♀}_{\text{small}} > \text{♀}_{\text{medium}} > \text{♀}_{\text{large}}$$

It therefore appears that size is the crucial determinant of these rates. For the male and female of equal size it is interesting that the male has a slightly higher rate of warming, and this may be attributed to the lower reflectance of males (section 4.2.1). The role of size is more clearly visible in Figure 4.6. Although none of the individual regressions are significant, if males and females are grouped the regressions are significant (warming: $y = 3.68 - 1.04x$, $r^2 = 0.724$, d.f. = 13, $p < 0.001$ and cooling: $y = 4.26 - 1.29x$, $r^2 = 0.719$, $p < 0.001$). Thus initial passive warming and initial passive cooling rates are inversely proportional to size. This is quantitative evidence to reinforce the more qualitative argument expressed in Figure 4.2.

The calculated warming and cooling rates for dried specimens represent an index of those rates that live individuals will experience in the field. The warming rates under the lamp will be indicative of warm up during basking in the absence of forced convection and LW radiation from the ground. The cooling rates will then

be indicative of a bee cooling passively in the shade, again without forced convection.

4.2.4 Live warm-up experiments

A series of tethered flight experiments were run using the set up detailed in section 2.10. Nothing other than passive warming was exhibited as the bees warmed up to room temperature. Furthermore it was never possible to induce flight in the tethered individuals in over 100 runs despite varying experimental conditions in an attempt to do so.

Upon capture, male and female *H. rubicundus* were handled as little as possible, kept in cool dark conditions during transportation and attached to the rig as quickly as possible. For several individuals the time between capture and testing was less than an hour. The initial temperature to which the bees were cooled was varied as was the position of thermocouple attachment or insertion and type of glue used. Some bees were left entirely undisturbed while attached to the thermocouple, while others were coaxed to fly by gently tapping the antennae or abdomen or gently blowing air over the head using a pipette. Even being taunted by harsh words from a frustrated biologist and the threat of an ethyl acetate jar did not do the trick.

It is assumed that this population (and maybe species) is not a suitable animal for these type of investigations. In the field this bee is noted for its extremely timid nature (relative to most other Hymenoptera) and has a habit of hiding in its burrow for long periods upon even a relatively mild disturbance (pers. obs.). Presumably the stress induced by capture, transportation and thermocouple attachment are too debilitating for the bee to behave in the same way as many other species do (Stone & Willmer 1989a).

In order to test whether the lack of flight activity was due to a fault in the experimental protocol, several other species (various *Bombus* sp., *Apis mellifera* and various *Andrena* sp.) were run on the apparatus, and both endothermic warm-up and flight were observed.

Thus it was not possible to obtain estimates of voluntary flight temperatures, stable flight temperatures and minimum flight temperatures in the laboratory.

4.3 Microclimate at the nest-site

4.3.1 Microclimatic variation on and above the nesting site substrate

The most important microclimatic factors affecting an insect's physiology and behaviour are temperature, solar radiation, relative humidity and wind velocity (Unwin & Corbet 1991). An example of microclimatic change through a day is given later in Figure 4.12. All of these parameters are inter-related and likely to vary greatly across the microhabitats used by *H. rubicundus*. Bees are found in three distinct microclimatic environments: flying close to the ground, basking on the ground, and within their burrows.

Since soil has a greater absorbency and specific heat capacity than air it will warm up quicker and reach a higher temperature under solar radiation; it will however be more prone to rapid fluctuations in temperature. T_a and wind velocity vary considerably with distance from the substrate and this gradient produces a boundary layer phenomenon (Willmer 1982). At ground level, wind velocity will be greatly reduced and T_a elevated; and reflected solar radiation and re-radiated LW radiation will become much more important factors in an insect's thermal balance (Casey 1981).

Solar radiation and T_a vary considerable through the day, and this is highly predictable in the absence of changing cloud cover and wind strength. For the day 14.8.92 (clear and with little wind) both parameters are conveniently modelled by a second order polynomial equation using time (t in hours BST) as the predictor. For T_a , $y = -30.96 + 7.39x - 0.23x^2$, $r^2 = 0.984$, d.f. = 13, $p = 0.405$ (Figure 4.12b) and for light intensity (L), $y = -2208.2 + 462.9x - 17.7x^2$, $r^2 = 0.995$, d.f. = 13, $p = 0.100$ (Figure 4.12c). The large p values from the runs test indicate that there is no significant deviation from the model.

Solar radiation is the primary determinant of ground temperature and T_a (Cloudsley-Thompson 1962). At the Invergowrie nesting site there is a close relationship between T_a , ground temperature (T_g) and nest entrance temperature (T_n) on a given day (Figure 4.7a). On this warm cloudless day the T_g excess above T_a increases as T_a increases, being about 8 °C at a T_a of 15 °C and increasing to about 14 °C at T_a of 30 °C. T_n also increases with T_a but a constant excess of 5 °C is maintained across a range of T_a . The general relationship between these variables on 14.8.92, remains unchanged across a variety of different weather conditions for other days. It follows that T_a will be a good predictor of T_g and T_n .

As the sun warms the ground and air through the day there is a strong positive relationship between L and T_a up to peak insolation levels (Figure 4.7b: $y = 0.028x + 2.345$, $r^2 = 0.952$, d.f. = 8, $p < 0.001$). T_a then remains roughly constant ($r^2 = 0.010$, $p = 0.850$) as L decreases through the afternoon.

Wind velocities (Wv) are greatly reduced at ground level: the mean Wv at 10 cm above ground for a 1 hour period on 21.6.95 was 1.1 m s⁻¹, but only 0.2 m s⁻¹ at ground level. There will therefore be reduced rates of convection on the ground, and this will be predominantly free rather than forced convection (Digby 1955).

The relatively low level of convection, in addition to the elevated temperatures on or near the ground, then provides a microhabitat for maximising rates of heat gain and minimising rates of heat loss.

4.3.2 Microclimatic variation within the nest

An insect's burrow may be subject to very different microclimatic conditions relative to the surface of the substrate. Changes in solar radiation through the day will cause the temperature to fluctuate greatly in the top few cm of soil. However at greater depths these fluctuations will be effectively 'damped' and thus a much more stable microhabitat will be maintained. The nest of *Cerceris arenaria* has an increasingly stable burrow temperature as depth increases (Willmer 1982).

The soil surface temperature at Invergowrie in Figure 4.8a is nearly 35 °C, and this temperature steadily decreases with depth to about 25 °C at 200 mm. The result of this temperature gradient is a soil moisture content gradient (Figure 4.8b). In the first 50 mm relative humidity (RH) increases markedly from 35 % to nearly 80 %. Deeper than this the RH increases only marginally with depth.

Developing eggs and larvae will have optimal temperature and RH requirements. In general, rates of development will increase with temperature up to a critical upper limit (Scriber & Slansky 1981); however drastic diel changes in temperature would not favour effective development, and would also risk damaging dehydration. Water loss is a problem for all developing bee larvae, and this has been partially overcome through the use of water resistant cell linings. The higher the RH of the surrounding soil (without waterlogging) the smaller the risk of desiccation. In the absence of other factors, a *H. rubicundus* female will then presumably select soil with a depth that provides the optimal conditions for her offspring. The usual depth of cells is about 50 mm (see section 3.4.3), and this

gives an apparently good compromise of warm temperatures (without too much daily fluctuation) with a relatively high humidity (Figure 4.8a and b).

The nest burrow temperature will be determined mainly by the surrounding soil temperature which in turn will depend upon the recent thermal history of the surface. Burrow temperature will inevitably be cooler than the soil temperature due to exposure to the air and evaporation at the soil surface. Nest temperature decreases with increasing depth (Figure 4.9a); so a range of thermal microhabitats is provided for a bee which could potentially be used for behavioural thermoregulation. Bees could use the warm burrow walls to elevate their body temperatures through conductive heat transfer. Indeed females are often found in the nest entrances apparently warming up prior to flight (see section 4.5.4), and this is especially common in the early morning or during cooler periods of the day (i.e. at low T_a).

Nests sited adjacent to stones had warmer nest entrances than those sited away from stones (Figure 4.9b), and this difference was significant (t-test: $T = 2.36$, d.f. = 17, $p = 0.030$). The stones on the ground surface at Invergowrie were very dark in colour and so likely to warm up faster than the surrounding soil in the morning. Females with burrows near stones would be able to warm up quicker and earlier in the day for their first emergence flights. This would give them a possible advantage in potentially increasing total foraging times by extending flight times into cooler periods of the day. Packer *et al.* (1989a) found that for three species of halictine bee in Nova Scotia, there was a preference for initiating burrows close to rocks and stones; where brood cells would experience elevated temperatures, and presumably faster developmental rates for the immatures.

4.3.3 Standard operative temperatures

T_{so} will normally be a complex function of L , T_a and Wv . However, under certain conditions T_{so} may be predicted by T_a during a single day (Figure 4.10a). On this clear and calm day, over 95 % of the variation in T_{so} ($r^2 = 0.959$, $p < 0.001$) is accounted for by variation in T_a . In this example T_{so} increases proportionally with T_a and maintains a constant excess of 4 °C above ambient (4.05 ± 0.23 °C (16)). T_{so} decreases as the height above the ground at which it is measured increases (Figure 4.10b). The greatest change is in the first few cm of height as the effect of the boundary layer is lost.

For T_{so} to be useful in predicting the behaviour and abundance of *H. rubicundus*, it needs to be applicable across a range of microclimatic conditions experienced throughout the flight season. As T_a varies with time of day and L , it is useful to quantify this for a number of readings made through days at various points in the season. Table 4.3 shows the results of the multiple regression analysis for T_a using L , time of day (t), Wv and RH as predictors. These variables explain 67.7 % of the variation in T_a ($r^2 = 0.677$, $p < 0.001$); and three of the terms were individually significant: L ($p < 0.001$), t ($p < 0.001$) and Wv ($p = 0.012$). As might be expected, T_a increases proportionally with L and t and inversely with Wv .

The relationship between T_a , L and t is represented graphically in the contour graph of Figure 4.11a. The boundary of this graph shows the range across which these variables were measured and the L and T_a gradients are clearly discernible. Maximum T_a s were recorded in the later parts of the day, but not always at peak L . This is most likely a sampling artefact since the days on which the observations were made did not include a period of high L late in the day (as observed on other non-recording days). If such a period had been included, then the graph

boundary would have been extended in its top right hand corner and would have included high T_a values.

When T_{so} was similarly analysed (Table 4.4), it was found that about 90 % of the variation in T_{so} could be accounted for by variation in the other microclimatic variables ($r^2 = 0.905$, $p < 0.001$). It is apparent that T_a and L alone are the two most important determinants of T_{so} (for both $p < 0.001$), and that the other variables are unimportant once the variation due to L and T_a has been removed (all with $p > 0.169$). Wv and t are not significant as their influence is already accounted for by T_a , since it is a function of these two variables anyway. Figure 4.11b clearly shows the influence of T_a and L on T_{so} ; with the highest values of T_{so} at maximum T_a and high L . Again the sampling procedure explains why T_{so} is not highest at maximum L on this graph.

T_{so} fails to take into account added convection due to flying, metabolic heat production and differences in mass. If these shortcomings are borne in mind, the sensibly applied use of T_{so} may serve as a good index for predicting the thermal status of an insect.

4.4 Abundance and microclimate

4.4.1 Microclimate, physiology and activity

Microclimatic parameters, and temperature especially, through their influence on physiology will determine activity patterns of insects through time. The power available from muscles will be an important determinant in the behavioural options open to an insect. Terrestrial locomotion is possible when muscles can only produce limited power, though the resulting movement may be relatively

slow. For flight, however, there is a threshold for power which is necessary for an individual to remain airborne.

Muscle performance of insects is strongly temperature-dependent (Josephson 1981) and the power generated during endothermic warm-up of bees (Stone 1993) and during flight is proportional to thoracic temperature. The ability of a particular individual to fly will then depend upon its body temperature (determined by size and ambient conditions, section 4.5.4) and so the proportion of a given population of insects that can fly is a function of the proportion that have thoracic temperatures above the threshold requirement.

The influence of various microclimatic factors upon insect activity has been widely studied (see section 1.1). Several authors (e.g. Juillet 1964; Burrill & Dietz 1981; Schöne & Tengö 1992) have measured the variation in the abundance of flying insects as a direct function of T_a and L (and sometimes RH and Wv); and others have additionally looked at the importance of the availability of floral rewards in these relationships (e.g. Willmer 1983; Stone 1994). The abundance of *H. rubicundus* females flying at the nest-site is presumed to be a good indicator of the level of foraging taking place at Invergowrie.

4.4.2 Abundance changes on a single day

The changes in relative abundance and microclimatic parameters through time on a typical warm and clear day at Invergowrie are given in Figure 4.12. Bee activity commences at about 08:30 and has completely finished by 16:30, and this is one of the longest recorded activity periods for *H. rubicundus* at this site. On cooler days activity started later and finished earlier with overall abundances being much lower; and when T_a failed to reach 14 °C bees never left their burrows. Once females begin foraging their numbers increase steadily until the middle of the day,

after which they decrease into the late afternoon (Figure 4.12a), and this unimodal pattern is unchanged throughout the season (subject to changes in ambient conditions). In the absence of microclimatic constraints female abundance coincides well with the peak pollen availability of *B. napus* and other floral resources (see section 3.5.1). Male abundance is also unimodal and closely follows the levels of female abundance (paired t-test: $T = 0.03$, d.f. = 28, $p = 0.970$); presumably this is a result of males coinciding their activity with females as they seek mating opportunities.

The variations in T_a , T_g , T_n and L through the day (Figure 4.12b and c) were discussed in section 4.3.1. RH will tend to be negatively related to T_a and L , and so will decrease through the day as the ground warms up (Figure 4.12d). The increase later in the day is probably a result of a drop in solar radiation added to the effect of moister air being brought in as W_v picks up after midday (4.12e). Activity dropped to virtually nothing around 30 minutes before the onset of rain and remained at this level even when other microclimatic conditions were suitable for flight. *H. rubicundus* females must be sensitive to some cue(s) in the environment (such as RH and/or pressure) that allows them to respond in anticipation to oncoming unfavourable weather (far more effective than local Meteorological office predictions).

As can be seen from Figure 4.13a, relative abundances for both sexes are predicted well by a quadratic function of T_a (females: $y = -0.262x^2 + 11.835x - 121.422$, $r^2 = 0.717$, d.f. = 13 and males: $y = -0.237x^2 + 10.240x - 96.948$, $r^2 = 0.827$, d.f. = 13) and neither deviated significantly from the model ($p = 0.100$ and $p = 0.916$ respectively). However L was not such a good predictor of abundance; and only when L was less than $\approx 650 \text{ W m}^{-2}$ was there any sort of proportional relationship between the two variables (Figure 4.13b).

4.4.3 Abundance changes on combined days through the season

In order for any model of abundance to be of more general use, it needs to combine data for a series of days across the season with different weather conditions. If abundance is plotted against T_a for several days (Figure 4.14a), the relationship is almost identical to that for a single day (females: $y = -0.154x^2 = 6.899x - 67.600$, $r^2 = 0.224$, d.f. = 47 and males: $y = -0.225x^2 = 9.412x - 85.428$, $r^2 = 0.146$, d.f. = 47) and neither deviated significantly from the quadratic model ($p = 0.129$ and $p = 0.051$ respectively). For both sexes, the amount of variation in abundance explained by T_a , for a single day, was more than 70 % but less than 25 % over a series of days. This decrease may be due to variation in other microclimatic parameters, such as cloud cover and wind, that remained relatively constant on 14.8.92, but may have caused rapid short term changes in abundance on other days.

Abundance varies proportionally with L (Figure 4.14b), although this is not initially apparent from the data due to the same sort of 'noise' described for T_a above. Consequently the amount of variation in abundance accounted for by variation in L is not high, especially for males (females: $y = 0.013x - 1.426$, $r^2 = 0.255$, d.f. = 48, $p < 0.001$ and males: $y = 0.012x + 1.824$, $r^2 = 0.078$, d.f. = 48, $p = 0.024$). High abundances are associated with low humidity levels (Figure 4.15a), owing to the approximately inverse relationship between RH and L . A W_v greater than 3 m s^{-1} was never recorded when bees were active at Invergowrie; for the majority of the time W_v was less than 1.5 m s^{-1} during bee activity (Figure 4.15b). There is no observable direct association between abundance and W_v , but W_v will have an indirect effect through T_a (see section 4.3.3).

Putting together the influences of light and temperature on abundance, a model can be drawn up. Although abundance and T_a have a curvilinear relationship, the

majority of abundance measurements were made at T_a s less than 23 °C (which are the normal conditions at Invergowrie). For this part of the curve (15 to 23 °C) the increase in abundance is roughly proportional to T_a . It may therefore be acceptable to enter this variable, along with L and t into a multiple regression as predictors for the response of abundance (Table 4.5). The results show a clear difference between the sexes. The abundance of males is clearly a positive function of L ($p = 0.001$) and T_a ($p = 0.012$), but independent of t ($p = 0.282$); and 75 % of the variation in abundance is explained by the first two variables. In contrast, female abundance is simply a function of L ($p < 0.001$) and not T_a ($p = 0.472$) or t ($p = 0.623$); and only 20.1 % of abundance variation is accounted for by L . Clearly the model would be improved by entering T_a as a quadratic term. The fact that for males T_a comes out as significant will be largely due to the influence of the seven relatively high abundances recorded below 23 °C, which will increase the linearity of the association at lower T_a s.

The overall relationship between abundance, L and T_a is presented in the contour graphs in Figure 4.16. The linear change in abundance above 700 W m⁻² is clear, as is the peak of the curvilinear T_a term at 21 °C. The similarity in the overall contour shape for males and females is striking. It is not, however, possible to determine whether this is due to both sexes' flight capabilities responding identically to microclimate characteristics, or whether male abundance is determined by female abundance *per se*. The former would seem unlikely as there is a large size difference between the sexes which inevitably results in very different body temperatures under a given set of microclimatic conditions (see section 4.5.4).

As an approximate predictor of female abundance T_{so} can be used (Figure 4.17). As only 14.9 % of abundance variation is accounted for by T_{so} variation, others factors must also be important. The minimum temperature necessary for flight is indicated by a T_{so} of ≈ 20 °C (see section 4.5.4E). There are few records of high

abundances below this critical T_{so} , and these are accounted for by observations made on a single day (22.7.92) when females had been prevented from foraging on the previous two days by heavy rain. The following clear day then presented the first opportunity with $T_{so} > 20\text{ }^{\circ}\text{C}$, which presumably led to increased pressure to forage and consequently high abundances. The equivalent T_a for a $20\text{ }^{\circ}\text{C}$ T_{so} is $16\text{ }^{\circ}\text{C}$ on a clear day (4.10a), and this T_{so} is represented by the area of $15 - 18\text{ }^{\circ}\text{C}$ T_a and $300 - 700\text{ W m}^{-2}$ on Figure 4.11a.

If figures 4.11b and 4.16b are compared visually it can be seen that the peak values are in different positions: maximum T_a for T_{so} and maximum L for female abundance. Overall, L alone is a better predictor of female abundance for this data set (Figure 4.14b). However the data collected here do not cover the entire range of microclimatic conditions that occur at Invergowrie; most notably T_a values of 22 to $28\text{ }^{\circ}\text{C}$ which are associated with L values greater than 800 W m^{-2} . It might be predicted that this area of the graphs would encompass peak T_{so} values and also peak female abundance. If this were the case then T_{so} and abundance would have a much stronger relationship, and consequently T_{so} would be of a much greater predictive value.

4.5 Field investigation of thoracic temperature

Section 4.4 demonstrated a clear link between key microclimatic parameters and the flight behaviour of *H. rubicundus*. The prime determinant in this relationship is the thoracic temperature (T_{th}) of the insect, which is subject to varying ambient conditions across the habitat and is also dependent upon the metabolic activity of the individual.

The collection of grab and stab data allows 'snapshots' of a bee's body temperature in the field to be ascertained for different activities under different

circumstances. Using a wide range of conditions allows analyses to be carried out to assess the importance of numerous individual influences on T_{th} , and further to look at the inter-relationships between several of these factors. Size, sex, microclimatic conditions and certain behavioural strategies are likely to have profound effects on T_{th} .

4.5.1 Analysis of ambient and ground temperature

In order to compare the T_{th} and T_{ex} of males and females while participating in different activities, it is necessary to examine the microclimatic conditions under which the data were collected, so that there is no bias within the different groups being examined. T_a was recorded for all the activities observed and T_g for the activities associated with the ground i.e. walking and basking (and just flying).

L was initially collected for all cases, but was found to be subject to too much extraneous variation to be of use in this kind of analysis. The readings of L , taken using a hand held solarimeter, varied greatly with slight changes in the angle it was positioned at (angle of incidence to incoming SW radiation); there was also the question of which angle to use. Ideally this would be parallel with the dorsal surface of the bee at the time of capture, but when basking this angle may be different to the ground angle, and it is completely unknown while flying. In addition, the full grab and stab process (capture, stab, measurement of T_{th} , T_a and T_g and recording of results) took in excess of 30 seconds, so that by the time L was measured it often did not represent the relevant value at the time when T_{th} was taken. Similar temporal variation existed with Wv since this could also not be measured quickly enough after the grab and stab took place. This variable seems to be of less critical importance as T_a takes into account some of the effect of Wv . For the abundance studies, L and Wv were measured from a stationary and horizontal position, this was however acceptable, as a mean value could be used

and the positioning of the instruments standardised across days. In a future investigation it would be preferable if a method could be devised to incorporate these factors into the grab and stab procedure, especially L as this seems to be an important determinant of T_{so} and abundance.

It was therefore decided that the analysis of T_{th} should concentrate on the two microclimatic variables of T_a and T_g . T_a was initially tested to see if there was any difference between the mean T_{as} for each activity recorded (Table 4.6a). A significant difference was found (oneway ANOVA: $F = 9.93$, d.f. = 86, $p < 0.001$), so a Tukey's pairwise comparison was carried out to determine for which activities T_a was dissimilar. The results of this are summarised in Figure 4.18a. The mean T_a at which flying T_{th} s were recorded is significantly bigger than the mean T_a for walking and basking. As flight can only occur at higher T_{as} it would be expected that this mean would be greater than when individuals are restricted to walking and basking, at lower T_{as} . There is no significant difference between the mean of just flying and the mean of basking T_{as} ; this is not surprising as basking is very often a prelude to flying, so these two activities are closely linked temporal variants of the same overall activity. It is likely that an individual that has just taken off has been basking previously, and has recently experienced very similar ambient conditions. On the other hand those individuals recorded as flying will include a significant number of individuals that have attained flying ability without the need to bask, so the mean T_a for flying records will be greater than for either basking or just flying. It is then reasonable to collapse these just flying and basking categories into a single behavioural category which will be termed 'basking' from now on. There is also no significant difference between the mean T_a for walking and basking. This is because at low T_{as} the bees will often walk on the banking surface before basking, often to search for a suitable site to bask (but see also 4.5.4E).

Finally, the three newly defined activity categories were further tested for differences in mean T_a due to sex (table 4.6b). From the results of the twoway ANOVA, it was found that there was no significant difference in the mean T_a for each sex ($F = 3.27$, d.f. = 1, $p = 0.075$) or for the interaction between sex and activity ($F = 1.02$, d.f. = 3, $p = 0.387$); but there was still a significant difference in the mean T_{as} of the three activities ($F = 10.77$, d.f. = 3, $p < 0.001$).

Using a similar twoway ANOVA for T_g (Table 4.7), there was no significant difference in the mean T_{gs} at which each sex was sampled ($F = 0.06$, d.f. = 0.06, $p = 0.802$), or the mean T_{gs} for the sex by activity interaction ($F = 0.40$, d.f. = 1, $p = 0.530$). There was however a highly significant difference in the mean T_{gs} at which walking and basking activity was measured ($F = 11.80$, d.f. = 1, $p = 0.002$), as is apparent from Figure 4.18b. This suggests that basking took place on areas of warmer ground and that bees would be forced to walk at lower T_{gs} in order to find more suitable spots to bask or to return to their burrows.

Therefore there is no bias in the T_{as} or T_{gs} at which the values for each sex were obtained and there is no significant interaction between sex and activity; however there are significant differences in the mean T_{as} and T_{gs} at which the various activities are recorded.

4.5.2 Analysis of size

Size will obviously have an important influence on the T_{th} and T_{ex} of an individual bee, so it is important that there is no bias in the head widths of individuals from any of the behavioural categories of each sex. Table 4.8 gives the results of the twoway ANOVA that tests for this. The difference in mean head widths of males and females is highly significant ($F = 77.54$, d.f. = 1, $p < 0.001$), as would be predicted from section 3.7.1. There is however no significant difference

in the head widths when activity is examined ($F = 0.45$, d.f. = 3, $p = 0.717$) or when the sex by activity interaction is examined ($F = 0.17$, d.f. = 3, $p = 0.916$). It is then fair to make comparisons within the various activity groups for each sex, as there is no bias in head widths.

4.5.3 Analysis of mean thoracic temperatures and mean temperature excesses

Having shown that there are no significant differences in ambient conditions or size between activity categories within each sex, it is then acceptable to make direct comparisons using the mean T_{th} s and T_{ex} s. The mean values of T_{th} , T_{ex} and T_a are summarised in Table 4.9. The results of the ANOVA carried out on T_{th} are given in Table 4.10a; there is a difference in the mean T_{th} s between sexes ($F = 4.63$, d.f. = 1, $p = 0.034$) and between activities ($F = 19.85$, d.f. = 2, $p < 0.001$) and there is also a significant interaction ($F = 4.27$, d.f. = 2, $p = 0.017$). If the sexes are grouped and the mean T_{th} s for activities as a whole compared using Tukey's pairwise comparison (Table 4.10b), then each mean is significantly different from the other two ($p < 0.05$ for all comparisons). The mean T_{th} for flying individuals is significantly greater than that of basking individuals which, in turn, is significantly greater than that of walking individuals. Finally using Tukey's honestly significant difference test (Pagano 1981), the mean T_{th} s are tested to see if there was a difference in the means between sexes within each activity category (table 4.9c). There is a significant sex difference ($p < 0.05$) for flying, with females having higher T_{th} s than males; but there is no significant difference between sexes for either walking or basking ($p > 0.05$). All of the above results are summarised in Figure 4.19a.

T_{ex} was analysed in an identical manner to T_{th} and the results are summarised in Table 4.11 and Figure 4.19b. There is no significant sex difference with all the

activities grouped ($F = 1.74$, d.f. = 1, $p = 0.190$) as was found with T_{th} . There is however a difference when the activities are broken down by sex, and the interaction effect between these two factors accounts for the lack of apparent difference when all activities are grouped i.e. the difference in male and female mean flying T_{ex} s is effectively cancelled out by an equivalent difference in the other direction for the mean basking T_{ex} s. The activities are also significantly different overall ($F = 13.38$, d.f. = 2, $p < 0.001$); with mean flying and basking T_{ex} being greater than mean walking T_{ex} ($p < 0.05$), and with there being no difference in the mean values for flying and basking ($p > 0.05$). For the two sexes combined, the mean T_{ex} of flying and basking individuals are approximately the same and both are greater than the mean T_{ex} for walking. Again the apparent lack of difference in the T_{ex} calculated for flying and basking results from the significant interactive effect of sex with activity ($F = 8.71$, d.f. = 2, $p < 0.001$). As with T_{th} , flying females had a significantly higher T_{ex} than males ($p < 0.01$), but there was no significant difference between sexes for either walking or basking behaviour ($p > 0.05$).

In summary, the relationships between and the relative magnitude of the mean T_{ex} and T_{th} values are very similar; this is as expected since $T_{ex} = T_{th} - T_a$, and T_a was previously shown not to be significantly different across the various categories (except for activity only). Any argument then applied to T_{th} will be equally applicable to T_{ex} and vice versa.

Females would in general be predicted to have higher overall body temperatures than males, owing to their greater size and consequently lower surface area to volume ratio (section 4.2). Ignoring the influence of sex, basking body temperatures (grouped mean = 24.6 ± 0.7 °C (37)) are very much greater than those when walking (grouped mean = 19.6 ± 1.3 °C (11)) and this will be due to three factors. Firstly basking was sampled at relatively higher ambient

temperatures than was walking (19.4 ± 0.4 °C (37) and 17.5 ± 0.9 °C (11) respectively); secondly basking individuals will be maximising the amount of solar radiation they are receiving through their posture in order to elevate T_{th} ; and thirdly walking individuals may be in a thermally unfavourable microhabitat and are thus walking in order to find a more suitable spot. The fact that mean T_{ex} of basking individuals (5.2 ± 0.4 °C (37)) is greater than that of walking individuals (2.1 ± 0.5 °C (11)) shows that as a behavioural strategy to increase T_{th} , basking is very effective.

With males and females grouped together, the mean T_{th} of flying bees (27.5 ± 0.6 °C (39)) is greater than that of basking bees (24.6 ± 0.7 °C (37)). Some of this difference is an inevitable consequence of the differences in T_a conditions during sampling (flying: 21.2 ± 0.3 °C (39), and basking: 19.4 ± 0.4 °C (37)). However the mean T_{ex} of flying bees (6.3 ± 0.5 °C (39)) is greater than that of basking bees (5.2 ± 0.4 °C (37)) although this difference is not quite significant. Therefore the net thermal gain in flying bees is greater than that in basking bees; it is not possible, however, to partition quantitatively the changes in the various modes of heat exchange that account for this (see also Appendix 1). It is reasonable to assume that LW radiative heat exchange is approximately constant for both activities, and so the differences must be due to changes in convection, radiative heat gain and metabolic heat production. A basking bee will principally gain heat through SW solar radiation and very little will be due to its metabolism (Leyton 1975); and convective (free) heat loss will be small as the bee is in the boundary layer (section 4.3.1). When flying, however, radiative heat gain will be somewhat reduced and metabolic heat production due to the inefficiency of flight will be high; convective (forced) heat loss, too, will be high. The net result of the relative changes in these three factors produces a greater T_{ex} when an individual elects to fly after basking.

There were no significant differences between male and female mean T_{th} s (and T_{ex} s) for either walking or basking behaviours. For both sexes though there was a marked increase in T_{th} between walking and basking activity (Figure 4.19a) and this can be explained as above. The difference is much more marked in males (walking: 17.5 ± 0.6 (5) °C and basking: 25.3 ± 0.9 °C (16)) than in females (walking: 21.4 ± 2.2 °C (6) and basking: 24.1 ± 0.9 °C (21)) and can be seen in Figure 4.19a. Within the boundary layer convective cooling will be much reduced due the lack of wind, and heat gain by solar radiation will be very important in a bees heat balance. The relatively high surface area of males allows them to absorb and emit radiation more quickly than females. Therefore a male will lose heat more rapidly while walking than a female would, and in addition the female's greater size and thermal inertia will result in her maintaining a higher T_{th} . Males spend longer periods basking (see section 4.5.4, for the behavioural explanation) and so achieve a T_{th} similar to that of females. The T_{ex} attained by males (6.2 ± 0.7 °C (16)) is greater than that attained by females (4.5 ± 0.5 °C (21)), and this is again due to the relatively longer bask times.

Females had significantly higher mean T_{th} s (29.4 ± 1.0 °C (19)) than males (25.8 ± 0.7 °C (20)) during flight; and the mean T_{ex} s achieved by females (7.8 ± 0.6 °C (19)) were also larger than those for males (4.9 ± 0.9 °C (20)). These differences correspond with the higher mass specific rates of convective heat loss that males will experience during flight. The difference in mean T_{th} between basking females and flying females is highly significant (Tukey's HSD test: $Q_{obt} = 6.12$, $k = 6$, $\alpha = 0.01$, $Q_{crit} = 4.99$, d.f. = 81, $p < 0.01$). This cannot be accounted for fully by the difference in the mean T_a s at which the samples were taken (flying: 21.6 ± 0.5 °C (19) and basking 19.6 ± 0.6 °C (21)), as this is only 2.0 °C increase in T_a for a 5.3 °C increase in T_{th} . Therefore the increase in metabolic heat production during flight must be far greater than the combined effect of increased convective heat loss and the slight decrease in radiative heat gain (Appendix 1). This change in thermal

balance is confirmed by the mean T_{ex} for flight also being significantly greater by 3.3 °C than the mean T_{ex} while basking (Tukey's HSD test: $Q_{obt} = 6.08$, $k = 6$, $\alpha = 0.01$, $Q_{crit} = 4.99$, d.f. = 81, $p < 0.01$).

The temperature changes in males are very different for the same basking to flying transition. There is no significant difference in mean male basking and flight T_{th} s (Tukey's HSD test: $Q_{obt} = 0.60$, $k = 6$, $\alpha = 0.05$, $Q_{crit} = 4.16$, d.f. = 81, $p > 0.05$); the actual difference being a 0.5 °C increase when airborne. The difference in T_{as} for basking (19.0 ± 0.3 °C (16)) and flying (20.8 ± 0.4 °C (20)) amounts to 1.8 °C. The net equivalent drop in T_{th} temperature is therefore 1.3 °C which is the difference in mean T_{ex} s during basking (6.2 ± 0.7 °C (16)) and flying (4.9 ± 0.5 °C (20)). This difference in means is not significant (Tukey's HSD test: $Q_{obt} = 2.27$, $k = 6$, $\alpha = 0.05$, $Q_{crit} = 4.16$, d.f. = 81, $p > 0.05$) with this data set however. The obligate warming due to muscle activity during flight must be offset by the disproportionately large increase in convective heat loss, which is also supplemented by a small decrease in radiative heat gain upon flight. All these factors are inevitable consequences of the relatively small size of males and the influence this has upon the bees thermal balance.

4.5.4 Relationships between behaviour, body temperature, size and the thermal environment

The preceding analysis has examined the differences in the mean body temperatures of males and females undertaking various activities. It is now prudent to look at how body temperature changes through a range of conditions, and how these relationships are influenced by the size of the bee. These findings can then be used to show why particular patterns of activity occur under given sets of conditions, and how body temperature is a key determinant of many aspects of the behavioural ecology of *H. rubicundus*.

A. Thoracic and ambient temperature

T_{th} during flight increases as T_a increases (Figure 4.20a) for both males and females. Some 46.3 % of male T_{th} variation but only 16.2 % of female T_{th} variation is accounted for by variation in T_a alone. If body temperature was being actively regulated then it would be expected that the gradient of these two regressions would be significantly less than one (May 1976) i.e. where $T_{th} = T_a$. The gradient for males is 1.05 ± 0.06 and for females is 0.92 ± 0.12 ; and both clearly include 1, so there is no indication of thermoregulatory ability across this range of T_a s. An analysis of covariance (ANCOVA), using T_a as a covariate, showed that there was a significant effect of T_a on T_{th} ($p = 0.002$) but no sex ($p = 0.639$) or T_a by sex interaction ($p = 0.833$) effect. Although there is no significant difference between the sexes, female T_{ex} is generally greater than the male T_{ex} , and this is presumably because of the lower surface area to volume ratios that females possess. Thus for any given T_a females have higher T_{th} than males (section 4.5.3). Further grab and stab data collected for females at lower T_a s would help clarify the relationship with T_{th} . There is a suggestion that female T_{th} may be more independent of T_a than is male T_{th} ; very little of the variation in female T_{th} is explained by variation in T_a and further the gradient of the line is marginally less different from zero than the male gradient.

T_{th} during basking increases with T_a , with both sexes maintaining a T_{ex} across the range of T_a s, and 54.4 % and 80.2 % of the variation in T_{th} is explained by variation in T_a for males and females respectively. The gradients of the two regression lines are both significantly greater than 1 (males: 2.14 ± 0.13 and females: 1.40 ± 0.03), thus indicating that basking at higher T_a s (corresponding to higher L_s) is more effective in elevating body temperature above ambient than at lower T_a s. This is the result of the increased warming effects of SW and LW radiation at higher T_a s. Heat loss by convection will increase across the range of T_a s measured here but

presumably not as quickly as radiative heat gain; and so as L (and therefore T_a) increases the amount of solar radiation and LW radiation from the ground that is available for absorption will also increase. The ANCOVA (with T_a as a covariate) confirms the significant effect of T_a on T_{th} ($p < 0.001$); the influence of sex ($p = 0.201$) and the interaction of T_a and sex ($p = 0.136$) are unimportant.

The change from basking to flight at a given T_a (compare figures 4.21a and b), confirms the T_{th} drop observed in males and T_{th} increase for females highlighted in the previous section. Overall, T_a is an important determinant of T_{th} for both basking and flying bees of both sexes. There are some notable differences in the form of the relationships and this is accounted for by the differences in size both within and between sexes. The importance of head width is therefore examined in the next section.

B. Temperature excess and size

Higher T_{ex} values are associated with larger head widths (i.e. larger body sizes) while bees are flying (Figure 4.21a). An ANCOVA (with head width as a covariate) shows that there is no significant effect of size ($p = 0.800$), sex ($p = 0.218$) or the interaction of size and sex ($p = 0.150$). When the two sexes are viewed together there is a very close overlap in the regression lines for the range of head widths shared in common. This is explained by the very similar surface area to volume ratio ($\approx 3 \text{ mm}^2 \text{ mg}^{-1}$) at the overlap in head widths. T_{ex} increases linearly with head width and is independent of sex; in the absence of significant radiative heat gain, convective heat loss is proportional to surface area and metabolic heat gain is proportional to thoracic mass, therefore there is no change in the form of the relationship across the entire range from small males to large females.

When the T_{ex} and head width relationship is examined for basking bees (Figure 4.21b) there is a positive relationship between these two parameters. Both regressions are significant and 39.0 % of male T_{ex} variation and 23.8 % of female T_{ex} variation is accounted for by head width variation. The effect of size on T_{th} is shown in the ANCOVA (using head width as the covariate): head width ($p = 0.014$), sex ($p = 0.191$) and head width by sex interaction ($p = 0.127$). However, in the area of size overlap, males are able to attain a much greater T_{ex} than females of equivalent size and this difference cannot be attributed to differences in surface area alone.

The most likely explanation is that males elect to bask for longer and this may be for two reasons: firstly they need to attain higher T_{th} s than females in order to achieve flight (see Figure 4.27 and associated discussion) or secondly they spend more time waiting on the banking than females. Males do in fact spend a great deal of time seeking mating opportunities with females, and since females are primarily concerned with foraging they waste little time on the banking surface as they enter and exit their nests quite rapidly (pers. obs.). Encounter frequency is often quite low (especially at low T_{as}) and so a sensible strategy for males would be to conserve energy by not flying continually, and instead remain on the banking in readiness for flight should a suitable encounter arise. Males that maintained a constantly high T_{th} through continued basking would have an advantage over those males that do not; it would not be unusual then for males to regularly 'overshoot' the minimum T_{th} required for flight. Since males undergo a net heat loss upon flying this may have an advantage in prolonging flight duration, especially under cooler conditions. Females on the other hand need only bask until they attain the required T_{th} and then immediately fly off as there is no advantage to them in remaining on the banking. Females also warm up once airborne and so the basking T_{th} temperature for females may not be quite as critical as it is for males. For a given size, males would be expected to have higher

T_{ex} s than females for behavioural rather than physical and/or physiological reasons.

Head width is a reasonably good predictor of T_{ex} (and T_{th}); however the strength of predictions is likely to be better for basking bees than for flying bees (comparison of r^2 and p values). The general observation that a males T_{ex} will decrease upon the initiation of flight and that a females T_{ex} will increase holds for any given T_{a} (compare 4.21a and b).

C. Basking body temperature and ground temperature

As T_{g} and T_{a} are closely linked it is fair to expect that the relationship between T_{g} and body temperature will be of a similar form. This is the case (Figure 4.22a and b), with T_{th} increasing proportionally with T_{g} for both sexes (males: $y = 0.760x + 6.328$, $r^2 = 0.851$, d.f. = 13, $p < 0.001$ and females: $y = 0.661x + 9.335$, $r^2 = 0.572$, d.f. = 13, $p = 0.001$). The variance in male T_{th} is better explained by variance in T_{g} (85.1 %) than it is by variance in T_{a} (54.4 %); whereas female T_{th} is less well explained by T_{g} (57.2 %) than T_{a} (68.8 %). The difference by sex is accounted for by males spending more prolonged periods on the ground than females, which remain on the banking surface only as long as is necessary (previous section). The improvement in the predictive value for males of using T_{g} instead of T_{a} is greater than the loss in the predictive value for females. The ANCOVA, with head width as the covariate, shows that there is a significant size effect ($p < 0.001$) but no sex ($p = 0.488$) or interaction ($p = 0.575$) effect. As expected T_{ex} also increases with T_{g} (Figure 4.22b. ANCOVA: T_{g} , $p < 0.001$; sex, $p = 0.775$; interaction, $p = 0.538$).

D. Predictions of thoracic temperature using size with ambient and ground temperatures

From the previous three sections it is most reasonable to use T_a and head width as explanatory variables for the T_{th} s of flying individuals; and T_g and head width for basking individuals. The results of the multiple regression for flight activity are given in Table 4.12. For males T_{th} is a positive function of T_a ($p = 0.004$) but independent of head width ($p = 0.336$); and 46.0 % of the total variation in T_{th} is accounted for by T_a . Even though head width was significant when used on its own, it is less important when combined with the influence of T_a , suggesting that there might be some relationship between these two variables. If head width is naively regressed against T_a there is no significant relationship ($r^2 = 0.029$, $p = 0.485$); however at lower T_a s only larger males may be able to fly (see next section). Therefore the head width influence may be important through the lower part of the T_a range and not so important in the rest. The graphical representation of the multiple regression is given in Figure 4.23a. There is an obvious T_{th} gradient from low to high T_a ; but there is no general gradient with the size axis. However if T_a s between 17 and 21 °C are examined then a T_{th} gradient is apparent, with the lowest T_{th} for bees with the smallest head widths. Maximum T_{th} (≈ 34 °C) for males occurs in large individuals at the highest T_a s.

If the same analysis is applied to flying females then T_a is highly significant ($p < 0.001$) and head width is almost significant ($p = 0.063$) with a total of 75.0 % of the variation in T_{th} explained. Applying the same reasoning used for males also reveals a T_{th} gradient at lower T_a s of 15 to 21 °C (Figure 4.23b). Peak T_{th} s (also ≈ 34 °C) of females were recorded for larger individuals at high T_a s. However, size is of greater predictive value for females than for males and this may be because of the relatively larger variation in the head widths in females. The increased

importance of head width in the overall regression equation for females results in a much bigger r^2 value than that obtained for males.

The regression undertaken for basking activity is summarised in table 4.13. For males 65.8 % of T_{th} variation was explained by the two predictor variables; however T_g was significant ($p = 0.005$) and head width was not ($p = 0.108$). Head width may be important at the lowest T_g s of 18 to 22 °C (Figure 4.24a); with the warmest baskers (≈ 34 °C) measured being medium to large in size and found at the highest T_g s. Some 87.4 % of female T_{th} variation was explained by the terms T_g ($p < 0.001$) and head width ($p = 0.163$); as with the other analyses, the temperature gradient is more important than the size gradient in predicting T_{th} . If Figure 4.24b is examined then there is some size gradient but it is rather fragmented.

Additional data would better resolve the importance of head width in determining T_{th} when considered in conjunction with ambient and ground temperatures. These equations all have reasonably good predictive powers as they explain between 46 % and 87 % of the total variation in T_{th} . This is unexpectedly good when the large array of possible influences are considered, and just two variables are found to be of such importance.

E. Behaviour, size and the thermal environment

Females will commence foraging as early as 08:30, providing that environmental conditions are suitable. The amount of provisions per cell and the total number of cells provisioned will depend upon the quantity of pollen collected; a female will then maximise her reproductive success by foraging as much as possible within the windows of pollen availability (see section 3.5.1). Since many days through the season have weather conditions that prevent foraging (≈ 25 % of days had no

bee activity at Invergowrie in 1994), it is advantageous for females to commence foraging as early as possible in the morning. T_a (and L) will be limiting factors early in the morning, so as these increase it would be expected that the largest females would be able to forage first as they would be able to maintain the minimum T_{th} for flight first. Head width and T_a at first emergence from the nest are indeed inversely correlated (Figure 4.25a: $y = -0.019x + 3.091$, d.f. = 13, $r^2 = 0.304$, $p = 0.027$). All females will warm up through conductive and radiative heat gain, but only the largest females will be able to fly continuously at lower T_{as} , as their rates of convective heat loss will be proportionally lower than the rates for smaller females.

Another approximate indicator of the importance of size in determining flight ability is given in Figure 4.25b. At the lowest T_{as} for each sex only the larger individuals in the population are observed flying; at higher T_{as} smaller individuals will also be able to fly continuously and so the size range observed will expand. By delimiting the T_a and size, estimated activity windows can be drawn up. These windows have the same general form, though the female window is shifted to lower T_{as} owing to their larger size.

In section 3.5.3 it was shown that forage trip duration decreased with increasing T_a (Figure 3.9a: $r^2 = 0.047$, d.f. = 108, $p = 0.023$). Temperate insects become more active as T_a increases (Linsley & McSwain 1956) and flight speed also increases. Therefore the time spent collecting the pollen and flying between the nest and flowers will be reduced. Additionally the need for basking to maintain flight capability will be diminished, and foraging trips at warmer T_{as} will presumably on average comprise fewer basking episodes and so will be shorter in total duration.

Bask duration on the ground surface for females decreases with increasing T_g (Figure 4.26a: $y = -1.161x + 61.75$, $r^2 = 0.426$, d.f. = 25, $p < 0.001$). At a T_g of 30.0 °C it is normal for 20 to 30 seconds to be spent basking, whereas at 45 °C this is reduced to 5 to 15 seconds. Presumably then the time required by females to attain a suitable T_{th} for flight by basking decreases with increasing T_g . If basking in the nest entrance is analysed then females also spend more time basking at lower T_n s (Figure 4.26b: $y = -14.361x + 349.64$, $r^2 = 0.177$, d.f. = 10, $p = 0.173$). Conditions that produce a T_a of 15 °C result in a T_g of ≈ 30 °C and a T_n of ≈ 20 °C (Figure 4.7a). The 20 to 30 seconds spent basking on the ground at this T_a is much less than the 60 to 80 seconds spent in the nest entrance basking. This may indicate the relative differences in the effectiveness of warming up by these two methods. Solar radiation during ground basking would appear to produce an elevated T_{th} for flight much more quickly than the conductive heat gain will during basking in the nest entrance. It might be expected then that females should bask on the ground near the nest rather than in the nest itself. However other behavioural factors are likely to account for why this does not occur; most importantly exposure to parasitic Diptera (section 3.8.1). By remaining in the nest a female effectively blocks parasite entry; and general exposure to other predators and parasites may therefore selected for the preference of basking within the nest. Females found basking on the ground had generally returned from foraging flights and were searching for their nests. Having just landed they have a slightly elevated T_{th} anyway, and so would not require such a long period of basking to resume flight. In the passive warming experiments of section 4.2, males took between 10 and 12 seconds to achieve a T_{ex} of 5 °C; whereas females took 12 to 15 seconds to reach the same T_{ex} . In these laboratory conditions, $Wv = 0$ and L was a constant 900 W m⁻²; consequently warm-up rate while basking in the field is likely to be much slower. Therefore when females bask on the ground they may not be achieving as high T_{ex} s as males as a result of the length of the bask.

Figure 4.27 summarises the T_a s at which various activities for both sexes were observed to occur. The minimum T_a for flight (MTFF) for females is about 14.5 °C and this agrees well with the observations made for the abundance studies (14.0 °C, figures 4.13a and 4.14a) and the activity window plot (15.0 °C, Figure 4.25a). The MTFF for males in Figure 4.27a is about 15.5 °C which is higher than that indicated by the abundance study (14.0 °C) but lower than the MTFF of the activity window plot (17.0 °C). The activity window plot used a only a small sample size and the data were not collected with any particular attention being paid to lower T_a s (as with the data collected for Figure 4.27). The data collected in the abundance study involved some very warm and clear days, when the early morning temperatures were quickly increasing; and the T_a of 14 °C was recorded some 15 to 20 mins before male abundance was actually assessed. T_a would have inevitably been higher by this point. In addition the MTFF is based on T_a alone, yet as was previously shown L is an important factor, and there may have been a relatively high L for the T_a of 14 °C, which combined to produce suitable conditions for flight. Overall then the MTFF shown by Figure 4.27 is probably the most reliable estimate of the true MTFF for both males and females.

The difference between male and female MTFF is a result of larger females being able to maintain a suitable T_{th} while flying due to their relatively smaller convective heat loss. Males tended not to be found flying so much below ≈ 17 °C. Under the range of conditions experienced at Invergowrie, there was no suggestion of an upper T_a limit for flight. Females were observed flying at 28°C (the warmest day during the study); however males were not recorded at such a high T_a . It would be expected that they would be more tolerant of high T_a s than females because of their smaller size and drop in T_{th} during flight. However their ability to attain higher T_{th} s than females when basking may be important. In Figure 4.20b, at a T_a of 20 °C females would be predicted to have a T_{th} of ≈ 25 °C and males a T_{th} of ≈ 28 °C. Male T_{th} increases faster than female T_{th} over a range

of T_a ; thus at a T_a of 25 °C females would have a T_{th} of 30 °C while the predicted value for males would be nearly 40 °C. Presumably such a high T_{th} must cause severe thermal stress, if not death, to a cool temperate bee. Although males may be able to fly at T_a s in excess of 25 °C, if they were forced to spend any amount of time on the ground surface (as is necessary for mating) they would risk excessively high T_{th} s. Alternatively males may choose to fly in cooler microclimates (such as further above the banking surface) and intersperse this with periods of apparent 'cooling off' deep in burrows, as was occasionally observed.

At T_a s of 14 to 17 °C males spend a large proportion of their time of walking on the ground surface looking for mating opportunities with females, as this is the only form of locomotion available to them. Above 17 °C, and throughout the lower part of the T_a flight range, males bask when necessary to allow them to continue flying. Females are found walking across a wide range of T_a s, because walking for females is nearly always associated with trying to locate their nest after returning from a foraging flight (females have no other particular reason to walk when they are unable to forage because of low T_a s). Females are also seen to bask throughout the range of T_a s at which they are able to fly; this is more common at lower T_a s when it is more often necessary to elevate T_{th} for flight.

4.6 Summary

H. rubicundus females are larger than males and correspondingly possess lower surface area to volume (mass) ratios resulting in slower rates of heat exchange. A female of mean size had a ratio of 2.6 mm² mg⁻¹ and a male of mean size a ratio of 3.5 mm² mg⁻¹. Where the size distributions of the two sexes overlap there found to be no difference in the surface area to mass between sexes (≈ 2.9 mm² mg⁻¹).

The reflectance of female thoracic cuticle (7.6 %) was slightly greater than that of males (6.7 %).

During the passive warming of live bees a T_{ex} was never achieved and further there is no significant difference in the cooling constants of live and dead individuals. This is suggestive of a lack of endothermic capabilities (see section 6.2). The values for conductance were $0.076 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ for females and $0.098 \text{ W g}^{-1} \text{ }^{\circ}\text{C}^{-1}$ for males; and were similar to other solitary bee species with comparable masses. Initial warming and cooling rates for both sexes were inversely proportional to body size, as would be expected from the Newtonian model.

The thermal properties of the ground surface and within the nest were highly dependent upon prevailing microclimatic conditions. A thermally heterogeneous environment at the nest-site provides the opportunity for behavioural thermoregulation through the utilisation of suitable thermal microhabitats. The siting of the nest close to a large stone may confer thermal advantages on the foraging female and her developing offspring.

The abundances of flying female and male *H. rubicundus* are highly predictable from microclimatic parameters (L and T_a); with predictions during a single day being more accurate than for those combining several days throughout the season. T_{so} data across the season are found not to be particularly useful in explaining female flight activity. Microclimatic conditions undoubtedly influence the flight activity in this species; however male abundance may be more directly determined by the abundance of females which in turn may be influenced by changes in the floral rewards available at the forage sites (see section 6.2).

Controlling for the influence of body size, it is possible to show that while basking, males are able to attain a $6.2 \text{ }^{\circ}\text{C}$ mean T_{ex} while females are able to

achieve 4.5 °C mean T_{ex} (1.7 °C less than males). This is accounted for by males being able to absorb more solar radiation per unit body mass by possessing greater relative surface area (helped possibly by lower cuticular reflectance) or by males spending longer basking. However once flight is initiated females warm up and maintain a 7.8 °C mean T_{ex} because their metabolic heat generation is greater than convective heat loss; while males cool down to a 4.9 °C mean T_{ex} owing to their greater heat loss due to convection being greater than their metabolic heat gain during flight. Thus on average females fly with T_{ex} s 2.9 °C higher than males.

The T_{th} and T_{ex} of both sexes depends on the prevailing T_a and also on the size of the individual while either basking or flying. Body temperatures increases with both T_a and head width. However if both these predictors are combined in a single model then size is only important at lower T_a s where its influence is greatest. The temperature at which females can emerge from their burrows to commence foraging is inversely related to their size, and the only the largest females are able to fly at the lowest T_a s.

Males are able to achieve a given T_{ex} more quickly than females owing to their body size; but tend to remain basking for longer periods than females do as they wait for mating opportunities. The length of time females bask for is inversely proportional to the temperature of the substrate.

Table 4.1. Cooling constants ($^{\circ}\text{C min}^{-1} ^{\circ}\text{C}^{-1}$) for live and dead bees. The r^2 values for the regressions of $\Delta T_{\text{th}}/\Delta t$ on $(T_{\text{th}} - T_a)$ are all > 0.926 and the associated p values are all < 0.02 .

<u>Bee</u>	<u>Head width</u>	<u>Dead</u>	<u>Alive</u>
Male 1	2.22	1.932	1.781
Male 2	2.34	1.635	1.818
Male 3	2.48	1.376	1.479
Male means:		1.647 ± 0.161	1.692 ± 0.108
Female 1	2.52	1.361	1.404
Female 2	2.63	1.295	1.334
Female 3	2.74	1.048	1.183
Female means:		1.235 ± 0.095	1.307 ± 0.065

Table 4.2. The mean initial warming and initial cooling rates ($^{\circ}\text{C s}^{-1}$) for three female and three male bees.

<u>Bee</u>	<u>Head width</u>	<u>Initial rates of</u>	
		<u>Warming (1 -10 $^{\circ}\text{C}$)</u>	<u>Cooling @ 5 $^{\circ}\text{C}$</u>
Male 1	2.22	1.40 ± 0.02 (30)	0.99 ± 0.04 (3)
Male 2	2.34	1.28 ± 0.02 (30)	0.89 ± 0.06 (3)
Male 3	2.48	1.06 ± 0.02 (30)	0.80 ± 0.02 (3)
Female 1	2.52	1.02 ± 0.02 (30)	0.73 ± 0.05 (3)
Female 2	2.63	0.93 ± 0.02 (30)	0.67 ± 0.02 (3)
Female 3	2.74	0.74 ± 0.01 (30)	0.46 ± 0.02 (3)

Table 4.3. Multiple regression analysis of ambient temperature. T_a = ambient temperature, L = light intensity, t = time, Wv = wind velocity and RH = relative humidity.

Regression equation:

$$T_a = -0.65 + 0.712 L + 1.41 t - 0.980 Wv + 0.0407 RH$$

$$r^2 = 0.677, p = 0.000$$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-0.652	2.418	-0.27	0.788
L	0.7123	0.1244	5.73	0.000
t	1.4051	0.1521	9.24	0.000
Wv	-0.9800	0.3774	-2.60	0.012
RH	0.04065	0.02923	1.39	0.169

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	4	669.70	167.43	40.81	0.000
Error	67	274.89	4.10		
Total	71	944.59			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
L	1	295.53
t	1	343.45
Wv	1	22.39
RH	1	7.94

Table 4.4. Multiple regression analysis of standard operative temperature. T_{so} = standard operative temperature, T_a = ambient temperature, L = light intensity, t = time, Wv = wind velocity and RH = relative humidity.

Regression equation:

$$T_{so} = 1.57 + 0.806 T_a + 0.625 L + 0.235 t - 0.309 Wv + 0.0334 RH$$

$$r^2 = 0.905, p = 0.000$$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	1.568	1.780	0.88	0.382
T_a	0.80625	0.08986	8.97	0.000
L	0.6254	0.1117	5.60	0.000
t	0.2348	0.1687	1.39	0.169
Wv	-0.3088	0.2913	-1.06	0.293
RH	-0.03339	0.02181	-1.53	0.131

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	5	1401.84	280.37	126.30	0.000
Error	66	146.51	2.22		
Total	71	1548.35			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_a	1	1265.77
L	1	120.28
t	1	5.38
Wv	1	5.20
RH	1	5.20

Table 4.5. Multiple regression analysis of relative abundance: (a) Females. (b) Males. Ab = abundance, T_a = ambient temperature, L = light intensity, t = time.

A. Female

Regression equation: $Ab = -0.01 + 0.222 T_a + 1.21 L + 0.285 t$, $r^2 = 0.201$,
 $p = 0.002$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-0.013	4.556	-0.00	0.998
T_a	0.2224	0.3073	0.72	0.472
L	1.2054	0.3273	3.68	0.000
t	0.2849	0.5771	0.49	0.623

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	3	494.04	164.88	5.71	0.002
Error	68	1959.96	28.82		
Total	71	2454.00			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_a	1	97.70
L	1	389.32
t	1	7.02

B. Male

Regression equation: $Ab = 10.7 + 1.34 T_a + 2.00 L + 1.06 t$, $r^2 = 0.750$,
 $p = 0.000$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	10.746	7.718	1.39	0.168
T_a	1.3408	0.5195	2.58	0.012
L	2.0000	0.5533	3.61	0.001
t	1.0584	0.9755	1.08	0.282

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	3	1134.37	378.12	4.59	0.006
Error	68	5600.25	82.36		
Total	71	6734.61			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_a	1	55.34
L	1	982.08
t	1	96.94

Table 4.6. (a) Oneway ANOVA on ambient temperature for four activities, and Tukey pairwise comparison for differences in ambient temperature between the four activities. **(b)** Twoway unbalanced ANOVA on ambient temperature for sex, activity and the interaction sex*activity using the general linear model (GLM).

A.

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Activity	3	152.65	50.88	9.93	0.000
Error	83	425.17	5.12		
Total	86	577.82			

Tukey's pairwise comparison

Family error rate = 0.05

Individual error rate = 0.0104

critical value = 3.71

Confidence intervals for the difference between column means and row means.

	Flying	Just flying	Walking
Just flying	-0.141 3.025		
Walking	1.729 5.783	0.121 4.506	
Basking	0.653 4.261	-0.973 3.003	-3.656 1.058

B.

<u>Source</u>	<u>d.f.</u>	<u>Seq SS</u>	<u>Adj SS</u>	<u>Adj MS</u>	<u>F</u>	<u>p</u>
Sex	1	12.154	16.312	16.312	3.27	0.075
Acivity	3	155.794	161.334	53.778	10.77	0.000
Sex*Act.	3	15.323	15.323	5.108	1.02	0.387
Error	79	394.546	394.456	4.994		
Total	86	577.817				

Table 4.7. Twoway unbalanced ANOVA on ground temperature for sex, activity and the interaction sex*activity using a GLM.

<u>Source</u>	<u>d.f.</u>	<u>Seq SS</u>	<u>Adj SS</u>	<u>Adj MS</u>	<u>F</u>	<u>p</u>
Sex	1	0.12	1.37	1.37	0.06	0.802
Acivity	1	253.46	253.46	253.46	11.80	0.002
Sex*Act.	1	8.64	8.64	8.64	0.40	0.530
Error	36	773.51	773.51	21.49		
Total	39	1035.74				

Table 4.8. Twoway unbalanced ANOVA on head width for sex, activity and the interaction sex*activity using a GLM.

<u>Source</u>	<u>d.f.</u>	<u>Seq SS</u>	<u>Adj SS</u>	<u>Adj MS</u>	<u>F</u>	<u>p</u>
Sex	1	1.79463	1.40131	1.40131	77.54	0.000
Acivity	3	0.02639	0.02446	0.00815	0.45	0.717
Sex*Act.	3	0.00926	0.00926	0.00309	0.17	0.916
Error	78	1.40961	1.40961	0.01807		
Total	85	3.23988				

Table 4.9. The mean thoracic temperature (T_{th}), temperature excess (T_{ex}) and ambient temperature (T_a) for the grab and stab data. All measurements in $^{\circ}\text{C}$, and numbers in parentheses indicate the sample size for all temperatures in that category.

<u>Activity</u>		<u>Flying</u>	<u>Walking</u>	<u>Basking</u>
Female	T_{th}	29.4 ± 1.0 (16)	21.4 ± 2.1 (6)	24.1 ± 0.9 (21)
	T_{ex}	7.8 ± 0.6	2.7 ± 0.8	4.5 ± 0.5
	T_a	21.6 ± 0.5	18.6 ± 1.6	19.6 ± 0.6
Male	T_{th}	25.8 ± 0.7 (20)	17.5 ± 0.6 (5)	25.3 ± 0.9 (16)
	T_{ex}	4.9 ± 0.9	1.4 ± 0.5	6.2 ± 0.7
	T_a	20.8 ± 0.4	16.0 ± 0.2	19.0 ± 0.3

Table 4.10. (a) Twoway unbalanced ANOVA on thoracic temperature for sex, activity and the interaction sex*activity using the GLM. **(b)** Tukey pairwise comparison test for differences in thoracic temperatures for the three activities. **(c)** Tukey HSD test for differences in thoracic temperatures between the sexes for each activity.

A.

<u>Source</u>	<u>d.f.</u>	<u>Seq SS</u>	<u>Adj SS</u>	<u>Adj MS</u>	<u>F</u>	<u>p</u>
Sex	1	40.381	69.64	69.64	4.63	0.034
Activity	2	591.09	597.00	298.50	19.85	0.000
Sex*Act.	2	128.34	128.34	64.17	4.27	0.017
Error	81	1218.05	1218.05	15.04		
Total	86	1977.87				

B.

Tukey's pairwise comparison

Family error rate = 0.05

Individual error rate = 0.0193

critical value = 3.37

Confidence intervals for the difference between column means and row means.

	Flying	Walking
Walking	4.629 11.281	
Basking	0.716 5.188	-8.350 -1.658

C.

Tukey HSD test

$\alpha = 0.05$ and $k = 6$.

<u>Activity</u>	<u>Q_{obt}</u>	<u>d.f.</u>	<u>Q_{crit}</u>	<u>Significant</u>
Flying	4.40	81	4.16	yes
Walking	2.36	81	4.16	no
Basking	1.37	81	4.16	no

Table 4.11. (a) Twoway unbalanced ANOVA on temperature excess for sex, activity and the interaction sex*activity using the GLM. **(b)** Tukey pairwise comparison test for differences in temperature excesses for the three activities. **(c)** Tukey HSD test for differences in temperature excesses between the sexes for each activity.

A.

<u>Source</u>	<u>d.f.</u>	<u>Seq SS</u>	<u>Adj SS</u>	<u>Adj MS</u>	<u>F</u>	<u>p</u>
Sex	1	8.228	10.257	10.257	1.74	0.190
Activity	2	155.179	157.444	78.722	13.38	0.000
Sex*Act.	2	102.573	102.573	51.287	8.71	0.000
Error	81	476.684	476.684	5.885		
Total	86	742.665				

B.

Tukey's pairwise comparison

Family error rate = 0.05

Individual error rate = 0.0193

critical value = 3.37

Confidence intervals for the difference between column means and row means.

	Flying	Walking
Walking	2.042 6.357	
Basking	-0.352 2.548	-5.272 -0.931

C.

Tukey HSD test

$\alpha = 0.05$ and $k = 6$.

<u>Activity</u>	<u>Q_{obt}</u>	<u>d.f.</u>	<u>Q_{crit}</u>	<u>Singnificant</u>
Flying	5.26	81	4.16	yes
Walking	1.26	81	4.16	no
Basking	3.12	81	4.16	no

Table 4.12. Multiple regression analysis of thoracic temperature during flight for: (a) Males and (b) Females. T_{th} = thoracic temperature, T_a = ambient temperature and HW = head width.

A. Males

Regression equation: $T_{th} = 18.4 + 0.934 T_a - 5.13 HW, r^2 = 0.460, p = 0.007$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	18.36	14.26	1.29	0.216
T_a	0.9341	0.2801	3.33	0.004
HW	-5.131	5.169	-0.99	0.336

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	2	71.373	35.686	6.81	0.007
Error	16	83.785	5.237		
Total	18	155.158			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_a	1	66.214
HW	1	5.159

B. Females

Regression equation: $T_{th} = -16.9 + 1.37 T_a + 6.38 HW, r^2 = 0.750, p = 0.000$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-16.935	7.630	-2.22	0.041
T_a	1.3679	0.2841	4.81	0.000
HW	6.378	3.193	2.00	0.063

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	2	249.94	124.97	24.03	0.000
Error	16	83.20	5.20		
Total	18	33.14			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_a	1	229.19
HW	1	20.74

Table 4.13. Multiple regression analysis of thoracic temperature during basking for: (a) Males and (b) Females. T_{th} = thoracic temperature, T_g = ground temperature and HW = head width.

A. Males

Regression equation: $T_{th} = -14.3 + 0.554 T_g + 11.0 HW, r^2 = 0.658, p = 0.002$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-14.27	14.08	-1.01	0.331
T_g	0.5536	0.1599	3.46	0.005
HW	11.025	6.355	1.73	0.108

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	2	138.252	69.126	11.54	0.002
Error	12	71.892	5.991		
Total	14	210.144			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_g	1	120.219
HW	1	18.033

B. Females

Regression equation: $T_{th} = -10.4 + 0.773 T_g + 6.23 HW, r^2 = 0.874, p = 0.000$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-10.37	11.41	-0.91	0.381
T_g	0.77348	0.08484	9.12	0.000
HW	6.234	4.189	1.49	0.163

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	2	288.10	144.05	41.68	0.000
Error	12	41.48	3.46		
Total	14	329.58			

<u>Source</u>	<u>d.f.</u>	<u>Seq. SS</u>
T_g	1	280.45
HW	1	7.65

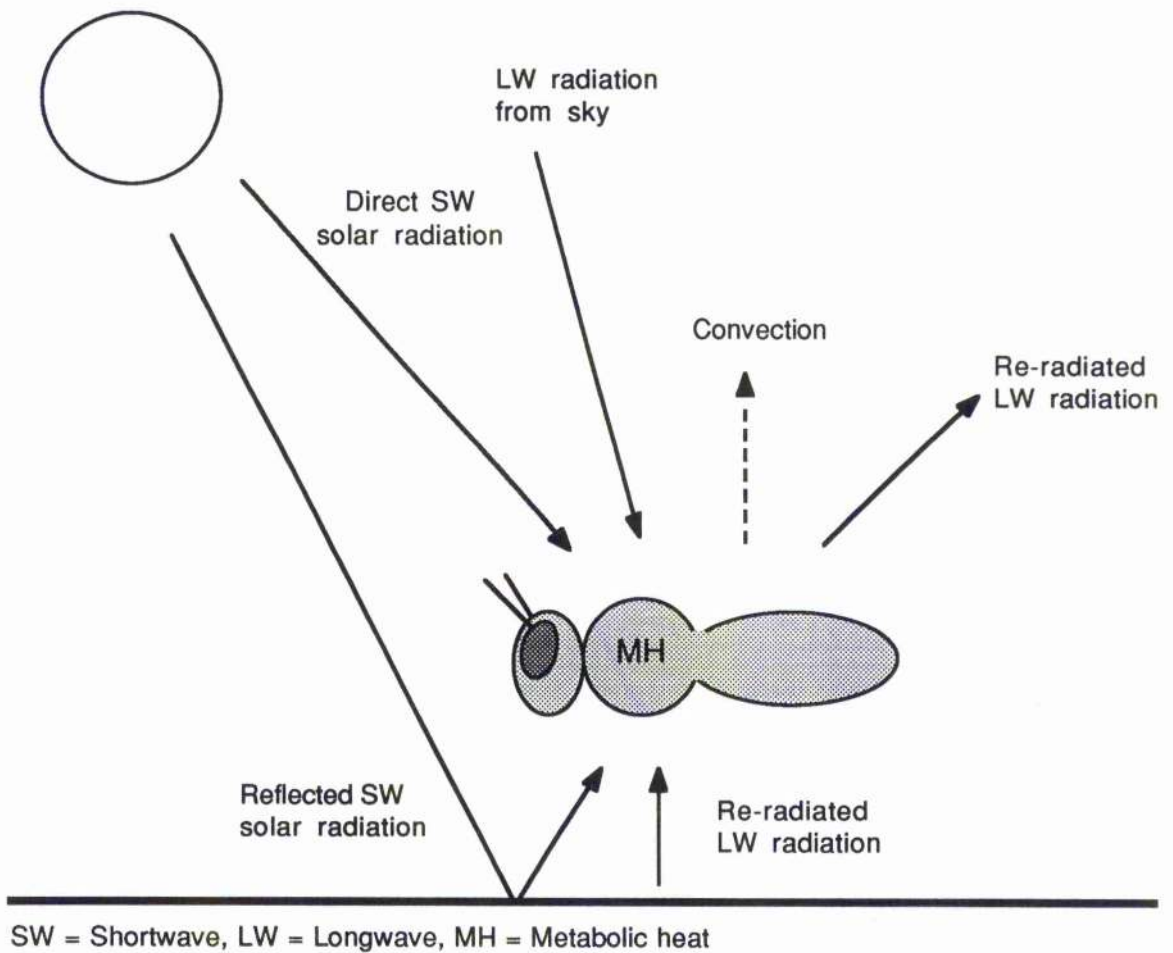


Fig. 4.1. Avenues of heat transfer between a bee and the environment. Evaporative and conductive heat loss are assumed to be negligible.

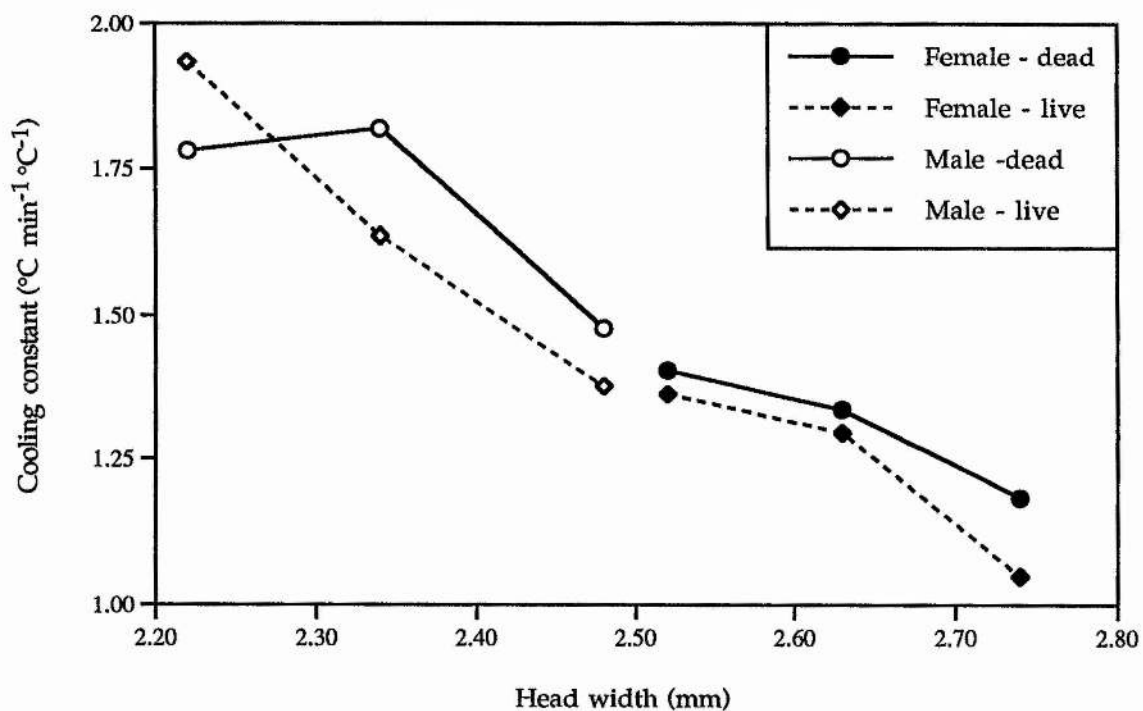
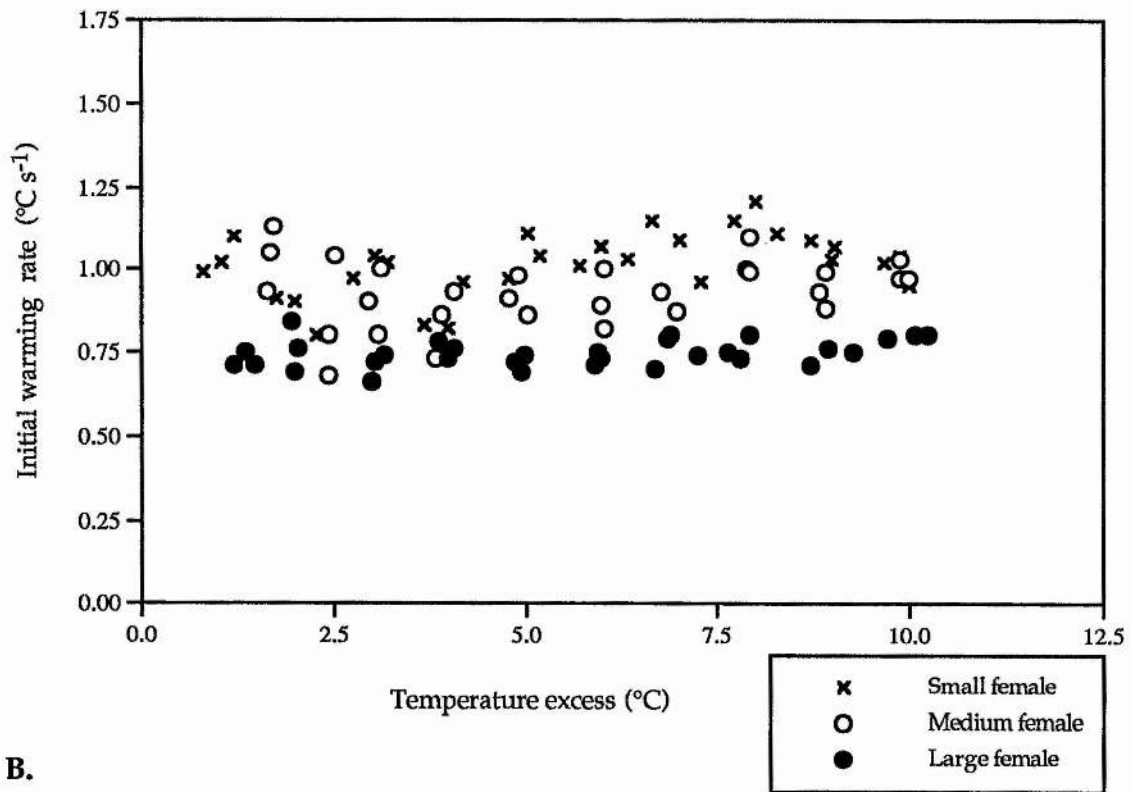


Fig 4.2. Cooling constants and head width for live and freshly killed bees while passively warming (see table 4.1).

A.



B.

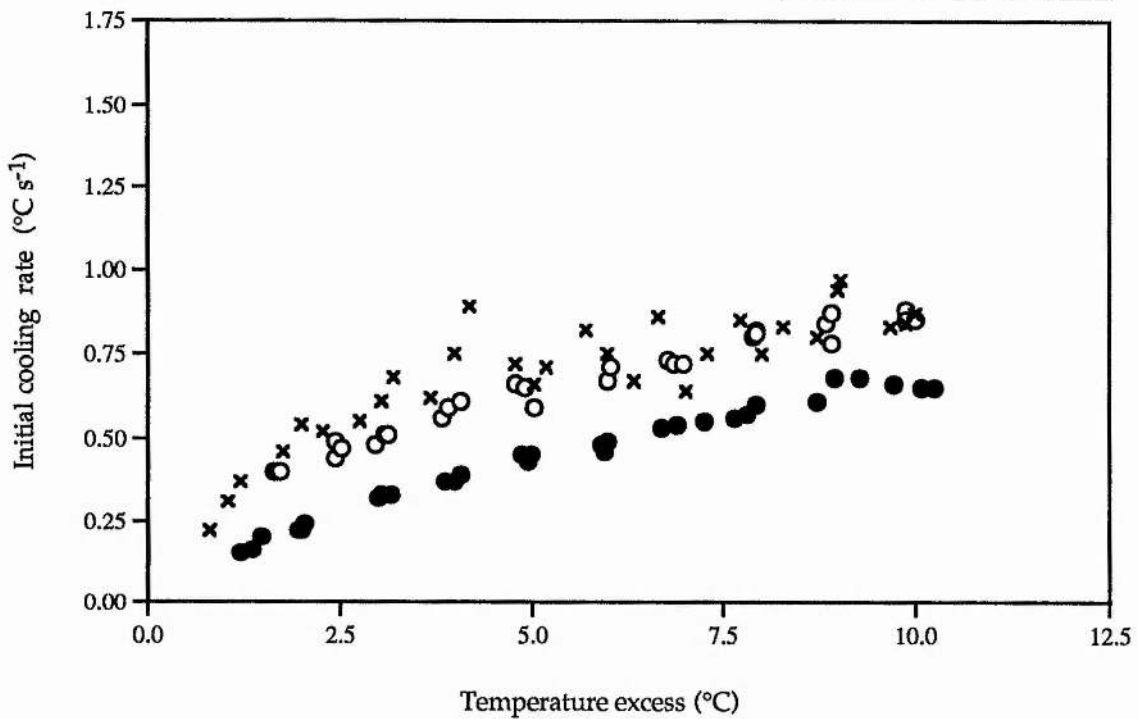


Fig 4.3. (a) Initial warming rates as a function of temperature excess for three females of different sizes. (b) Initial cooling rates as a function of temperature excess for the same three females. The live mass for the small female was 22.0 mg (2.52 mm), for the medium female 30.1 mg (2.63 mm) and for the large female 37.7 mg (2.74 mm).

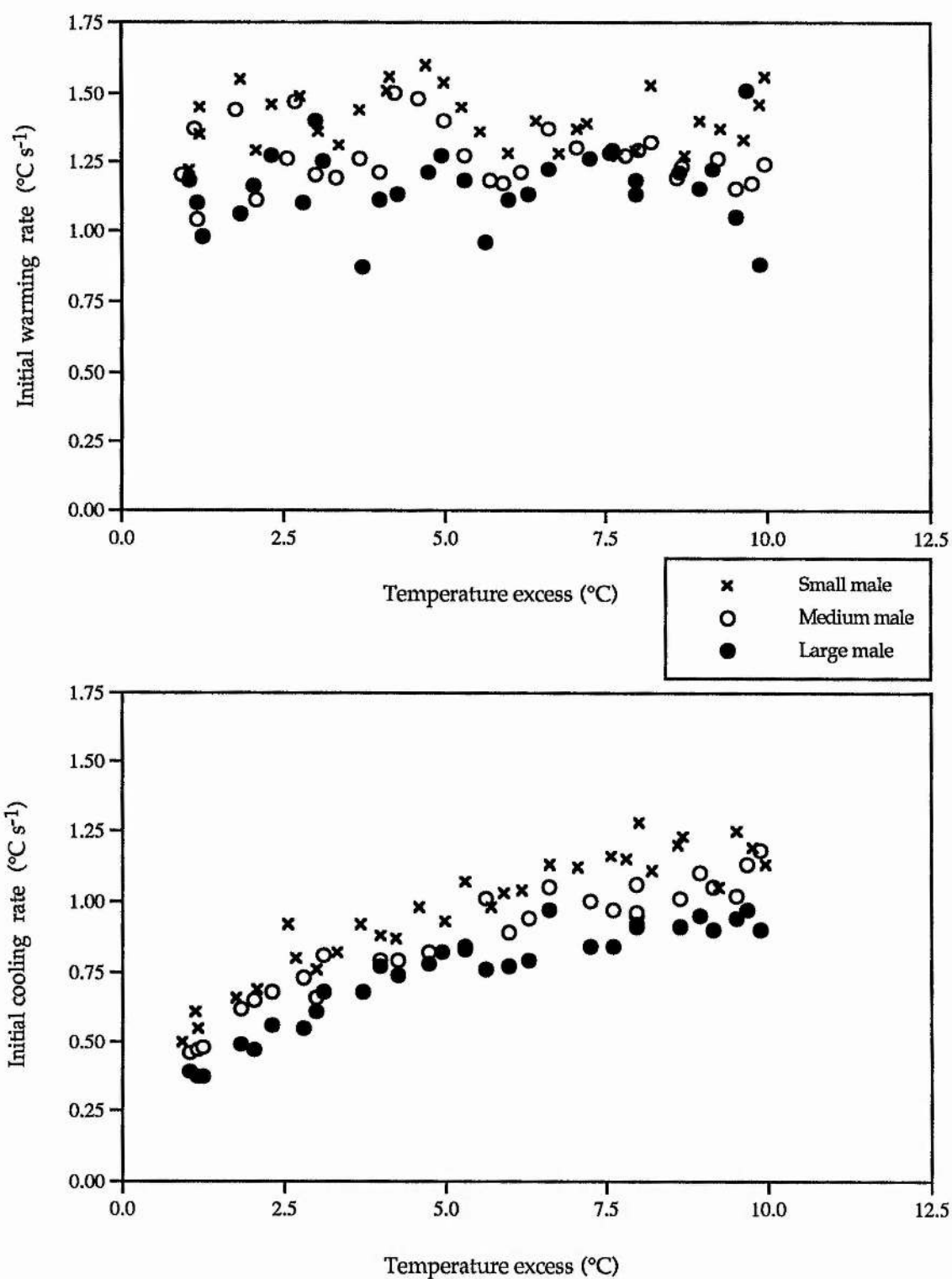
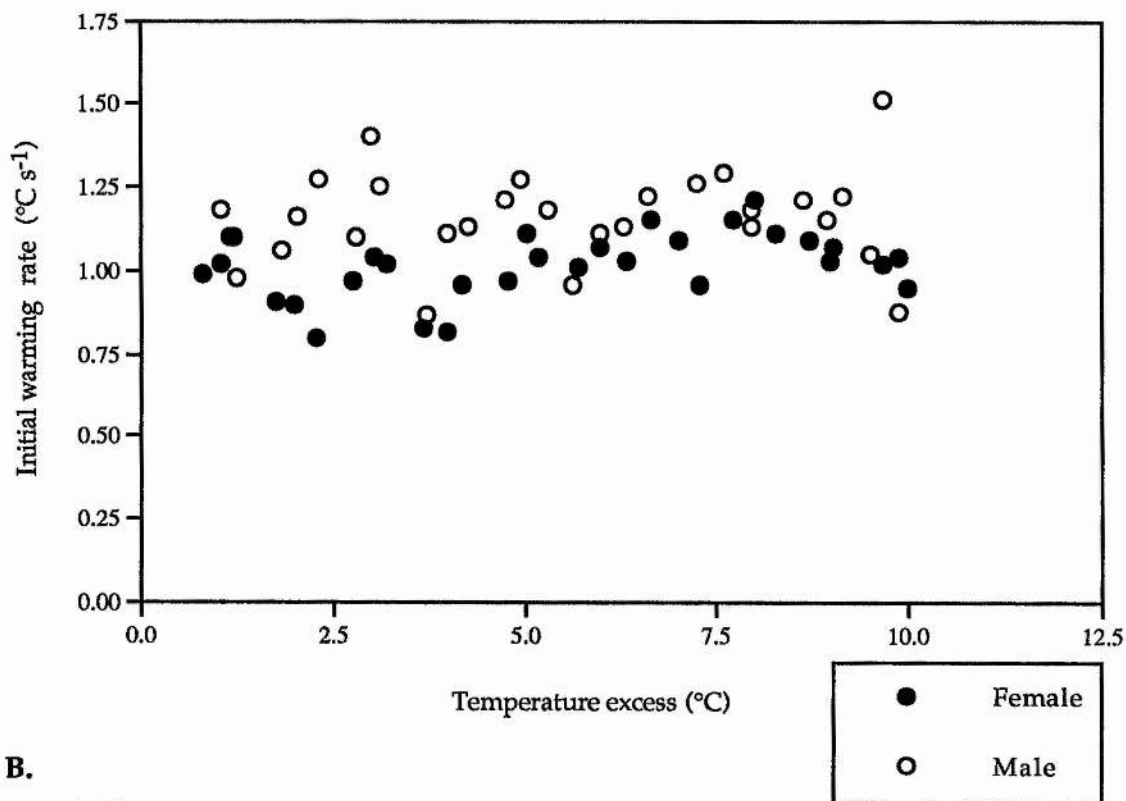


Fig. 4.4. (a) Initial warming rates as a function of temperature excess for three males of different sizes. (b) Initial cooling rates as a function of temperature excess for the same three males. The live mass for the small male was 10.7 mg (2.22 mm), for the medium male 16.1 mg (2.34 mm) and for the large male 22.5 mg (2.48 mm).

A.



B.

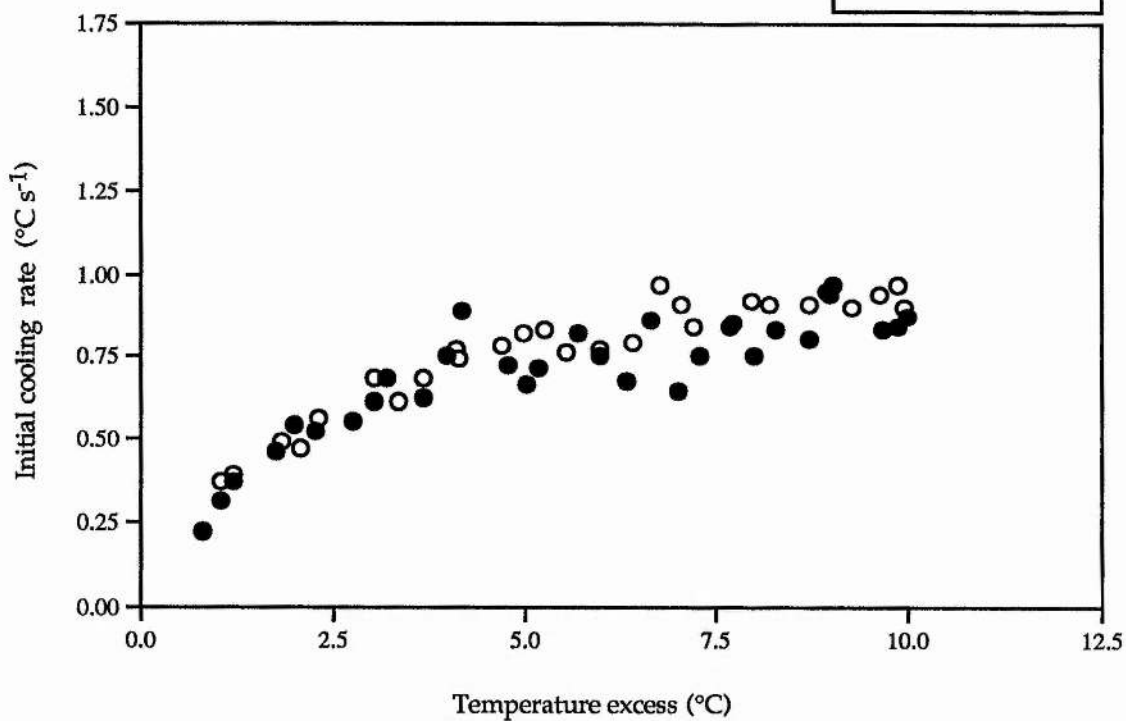
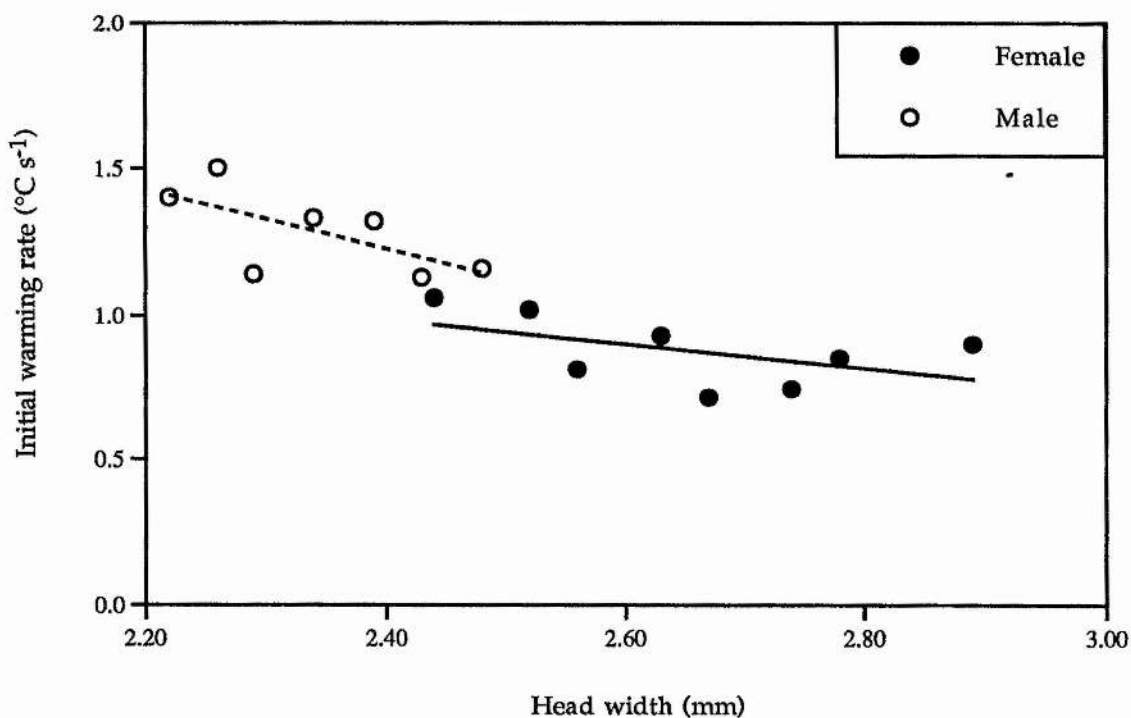


Fig. 4.5. (a) Initial warming rates as a function of temperature excess for a male and female of equal size. (b) Initial cooling rates as a function of temperature excess for the same individuals. The live mass for the male was 22.5 mg (2.48 mm) and for the female was 22.0 mg (2.52 mm). Numbers in parentheses are head widths.

A.



B.

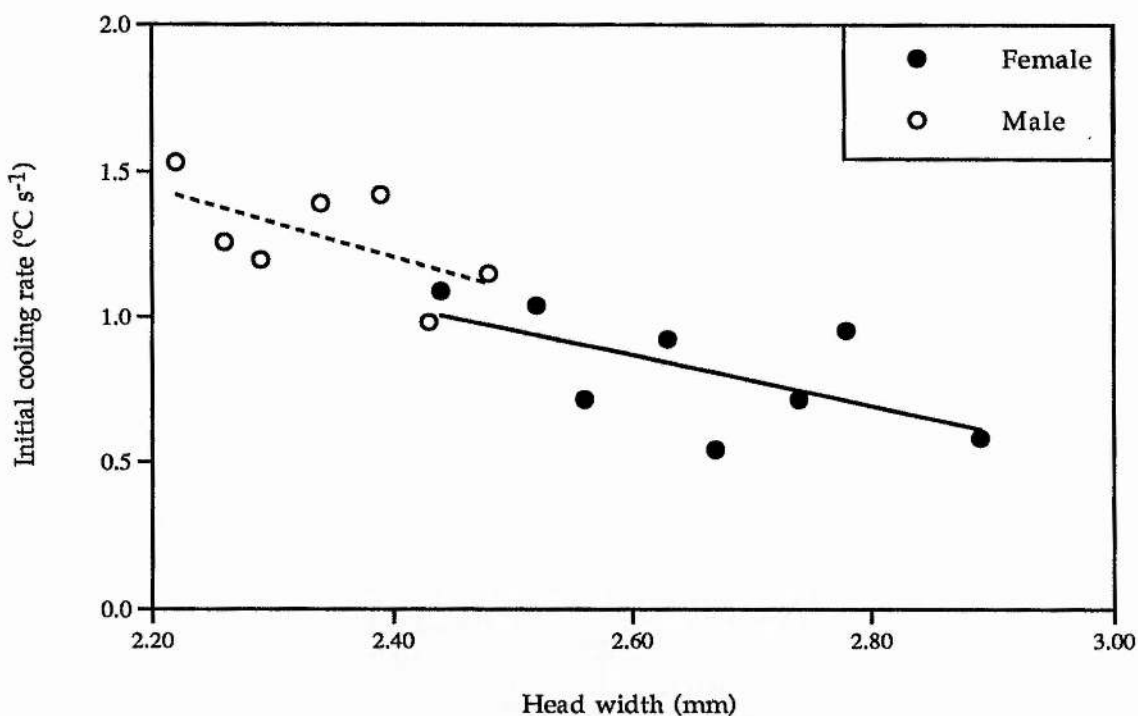
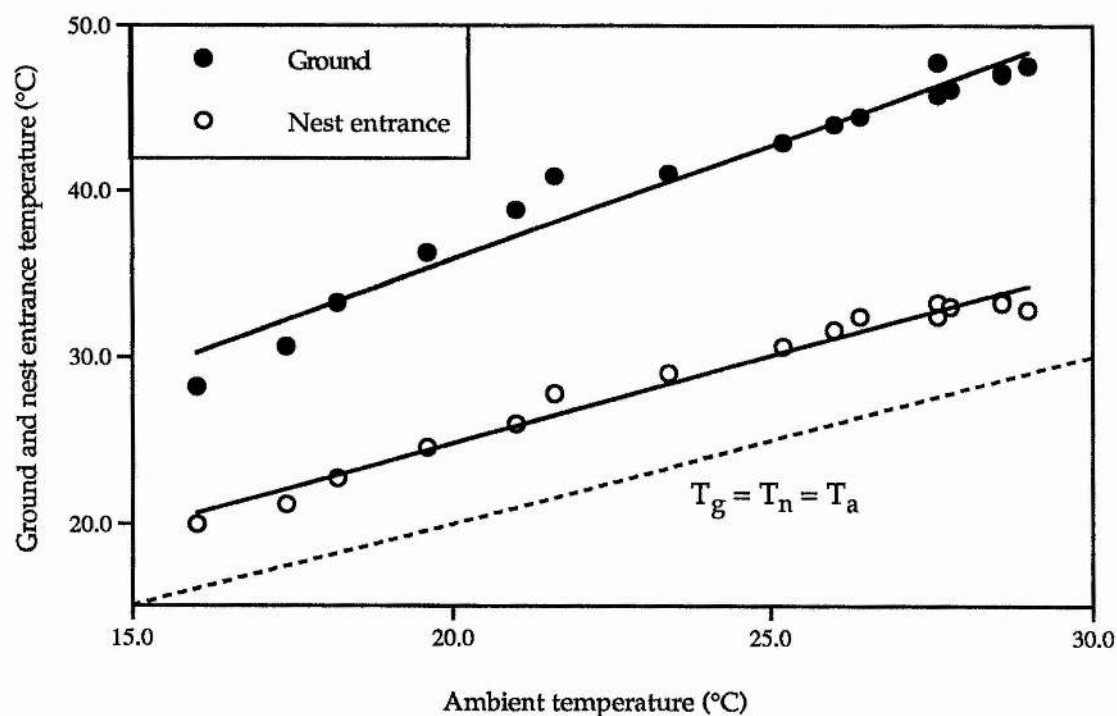


Fig. 4.6. (a) Initial passive warming rates as a function of head width for females ($y = -0.431x + 2.022$, $r^2 = 0.260$, $p = 0.197$) and males ($y = -1.000x + 3.628$, $r^2 = 0.433$, $p = 0.108$). (b) Initial passive cooling rates as a function of head width for females ($y = -1.171x + 4.020$, $r^2 = 0.352$, $p = 0.160$) and males ($y = -0.876x + 3.143$, $r^2 = 0.379$, $p = 0.104$).

A.



B.

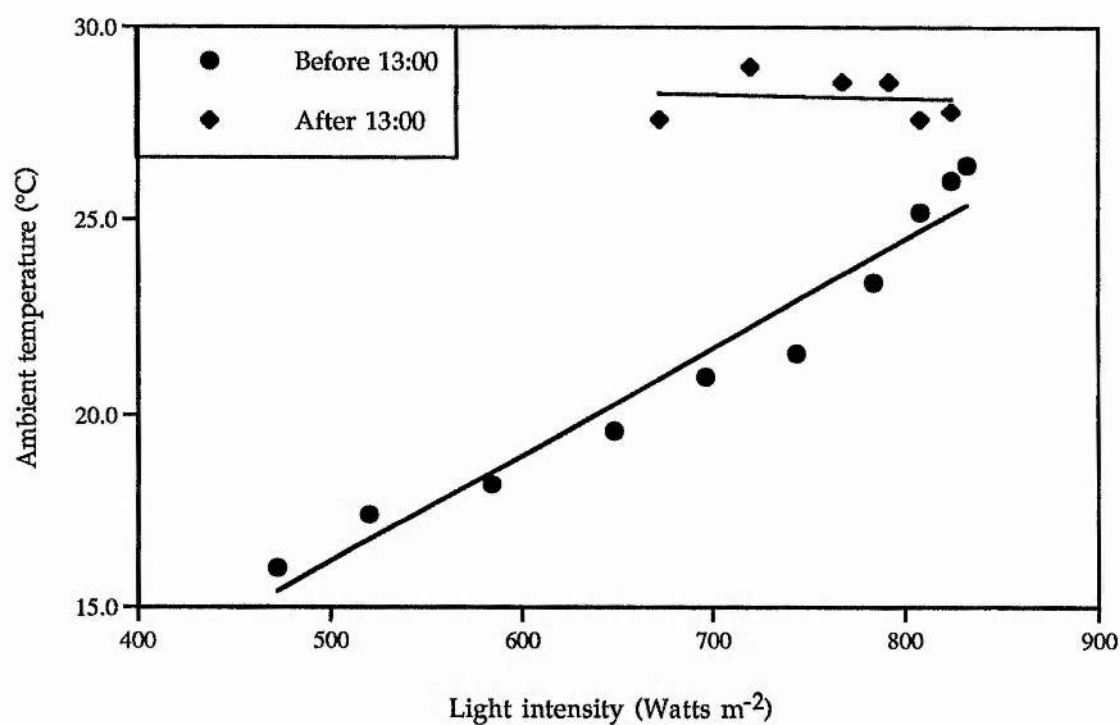


Fig. 4.7. (a) Variation in ground temperature ($y = 1.402x + 7.766$, $r^2 = 0.963$, d.f. = 14, $p < 0.001$) and nest entrance temperature ($y = 1.043x + 3.980$, $r^2 = 0.977$, d.f. = 14, $p < 0.001$) with ambient temperature. (b) Ambient temperature and light intensity: before 13:00 ($y = 0.028x + 2.345$, $r^2 = 0.952$, d.f. = 8, $p < 0.001$) and after 13:00. All measurements taken on 14.8.92 at Invergowrie.

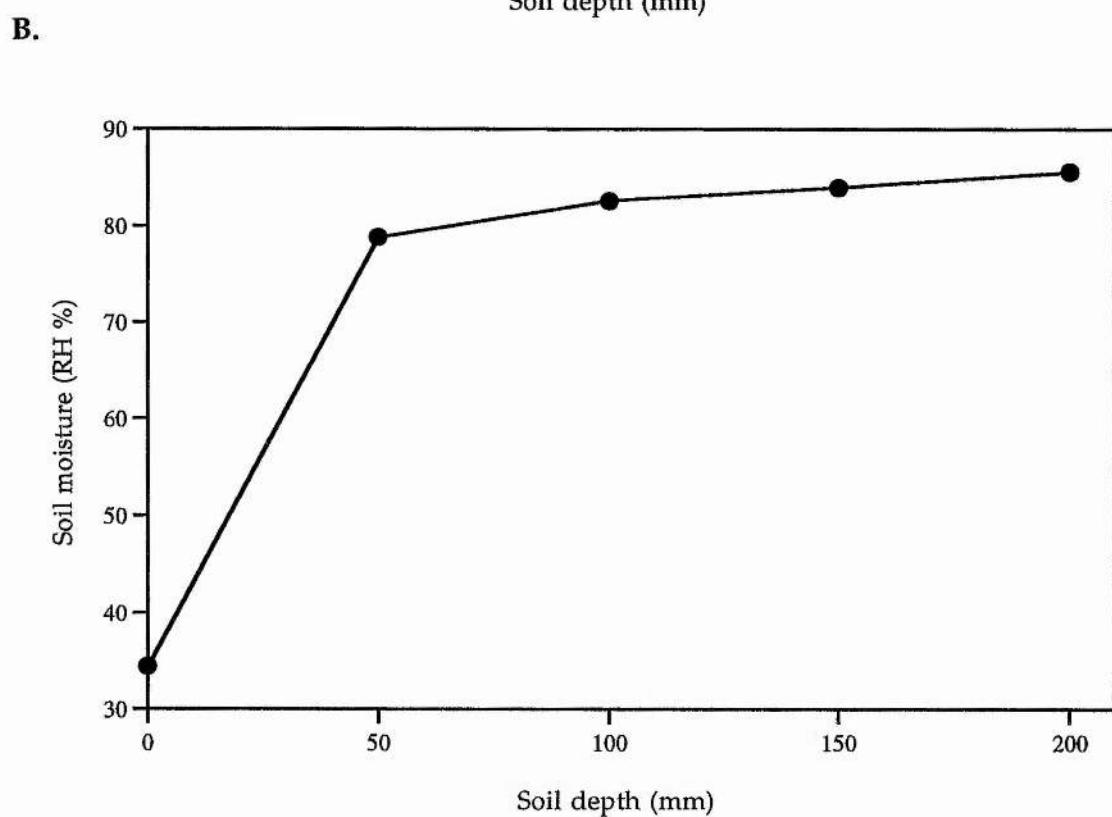
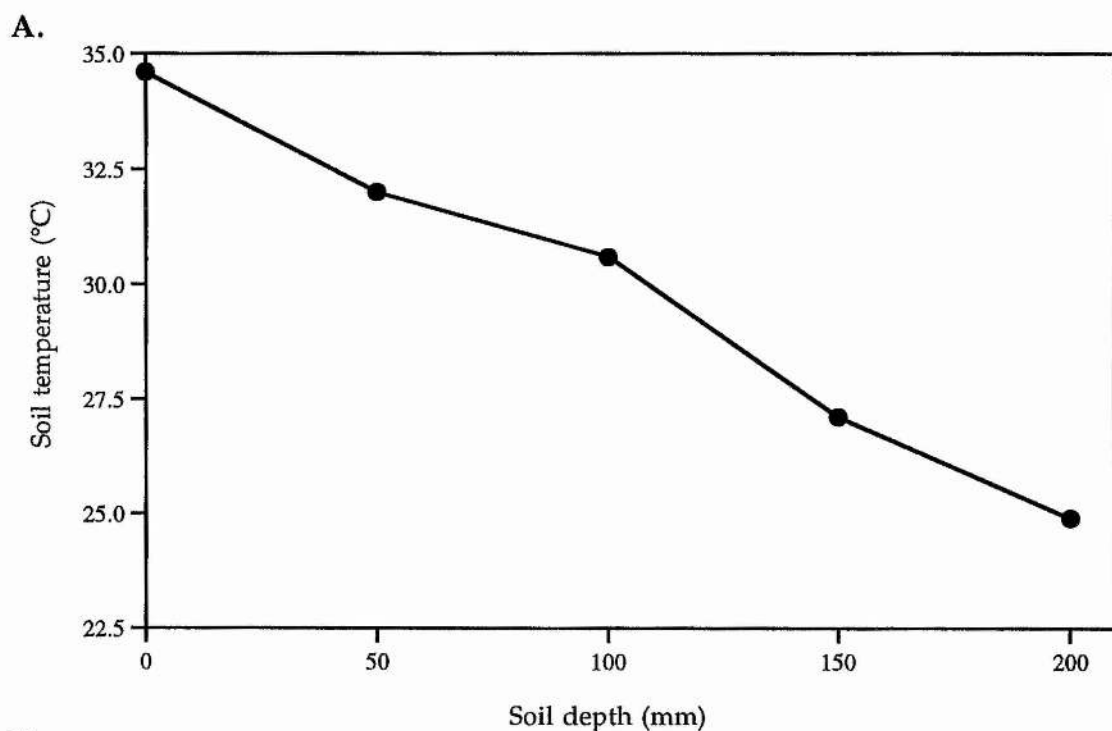
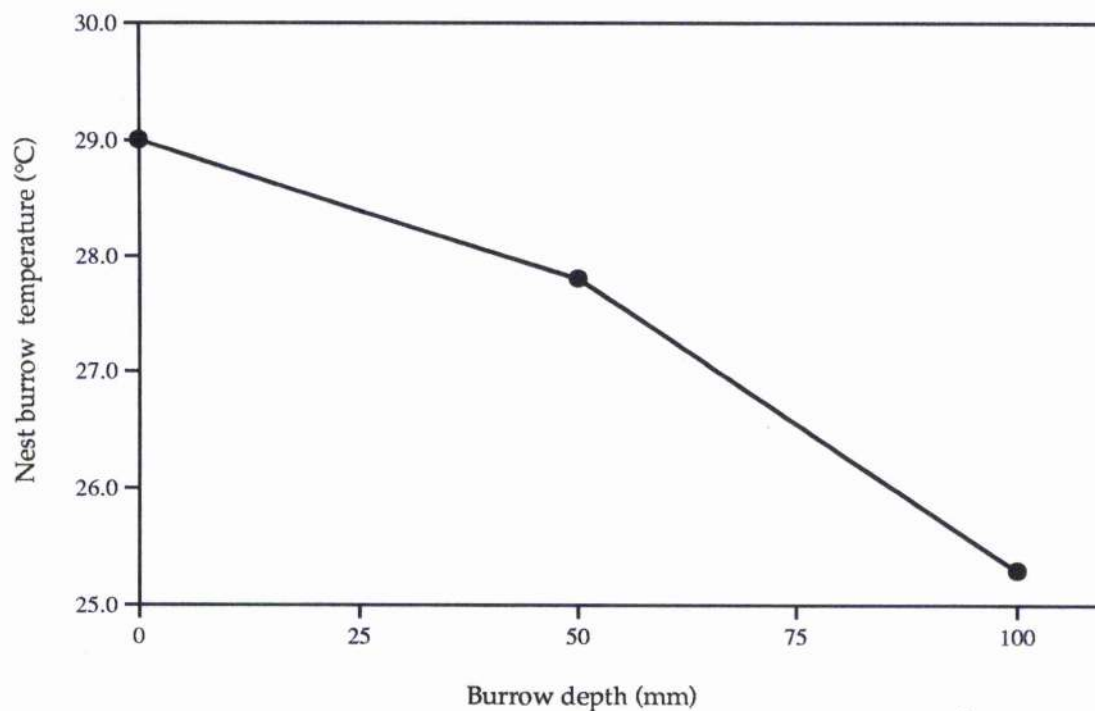


Fig. 4.8. Temperature and soil moisture profile for quadrat 41B at Invergowrie. **(a)** Soil temperature and depth. **(b)** Soil moisture and depth. Measurements made at 13:30 on 30.7.94 ($T_a = 22.8^\circ\text{C}$ and $\text{RH} = 52.6\%$).

A.



B.

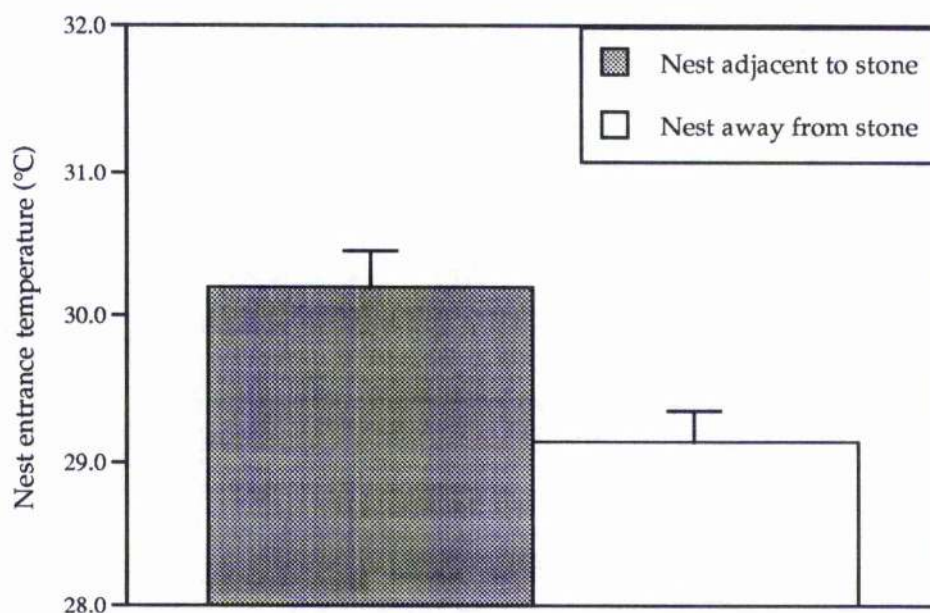
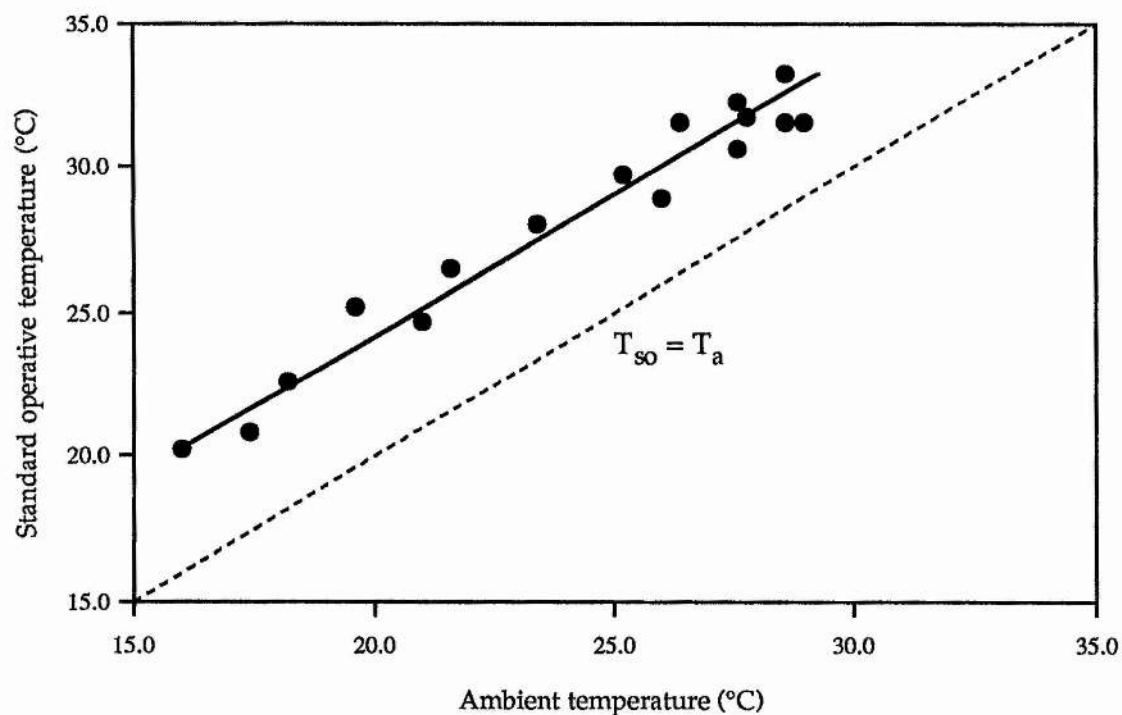


Fig. 4.9. (a) Burrow temperature as a function of nest depth. Readings made at Invergowrie, at 15:00 on 17.7.92 ($T_a = 25.4$ °C, $T_g = 37.8$ °C, $L = 776$ W m⁻², RH = 30.9 %, $Wv = 1.76$ m s⁻¹). **(b)** Mean nest entrance temperatures for 10 nests adjacent to a stone (> 5 mm diameter) and 10 nests away from a stone. Two sample t-test: $T = 2.36$, d.f. = 17, $p = 0.030$)

A.



B.

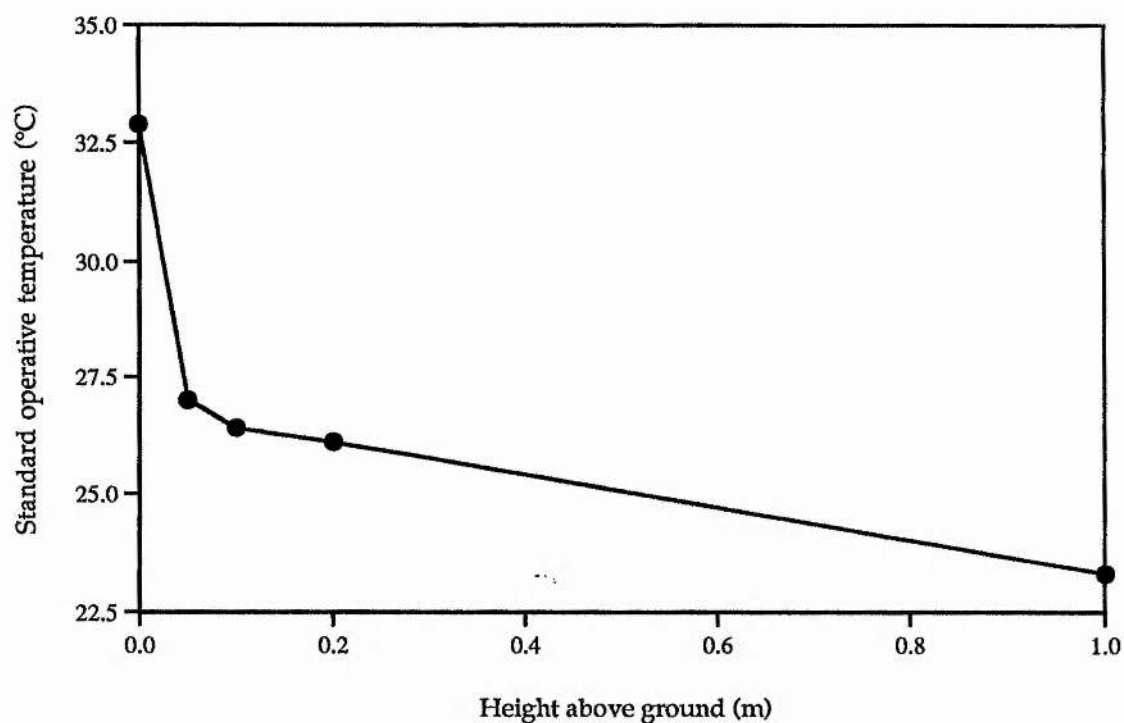
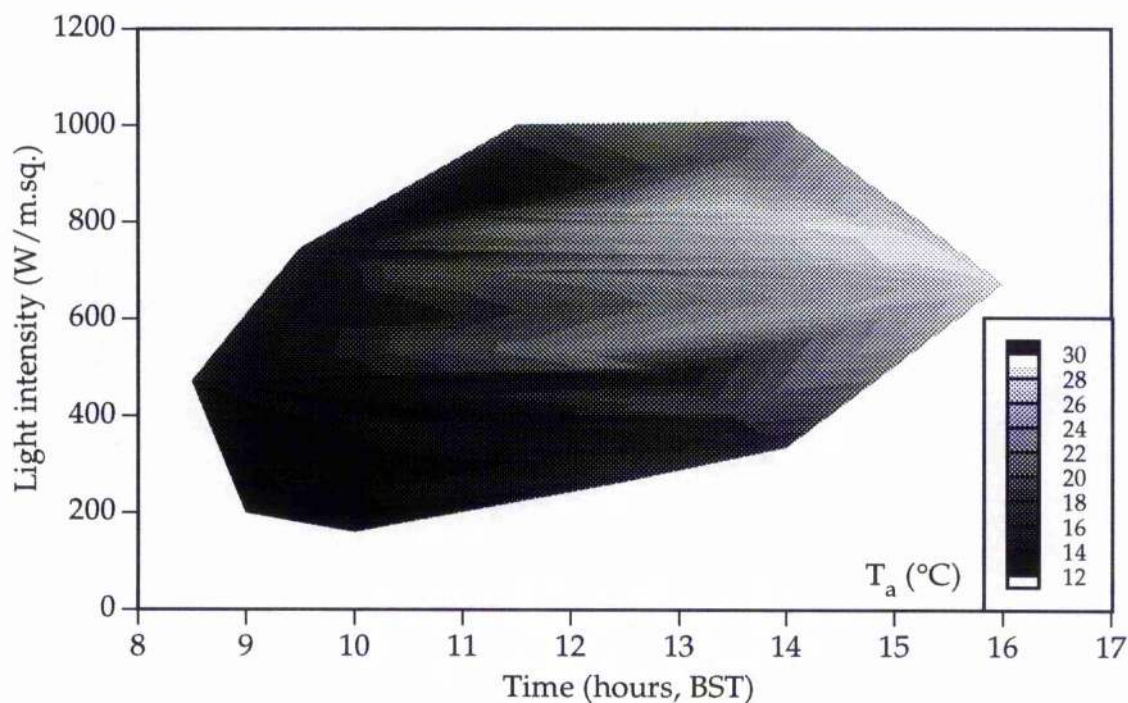


Fig. 4.10. (a) Standard operative temperature and ambient temperature measured at 100 mm above the ground at Invergowrie on 14.8.92 ($y = 5.61 + 0.935x$, $r^2 = 0.959$, d.f. = 14, $p < 0.001$). (b) Standard operative temperature as a function of height above the ground. Readings taken at Invergowrie, at 15:00 on 28.7.92. ($T_a = 25.4^\circ\text{C}$, $T_g = 37.8^\circ\text{C}$, $L = 776 \text{ W m}^{-2}$, $\text{RH} = 30.9\%$, $Wv = 1.76 \text{ m s}^{-1}$).

A.



B.

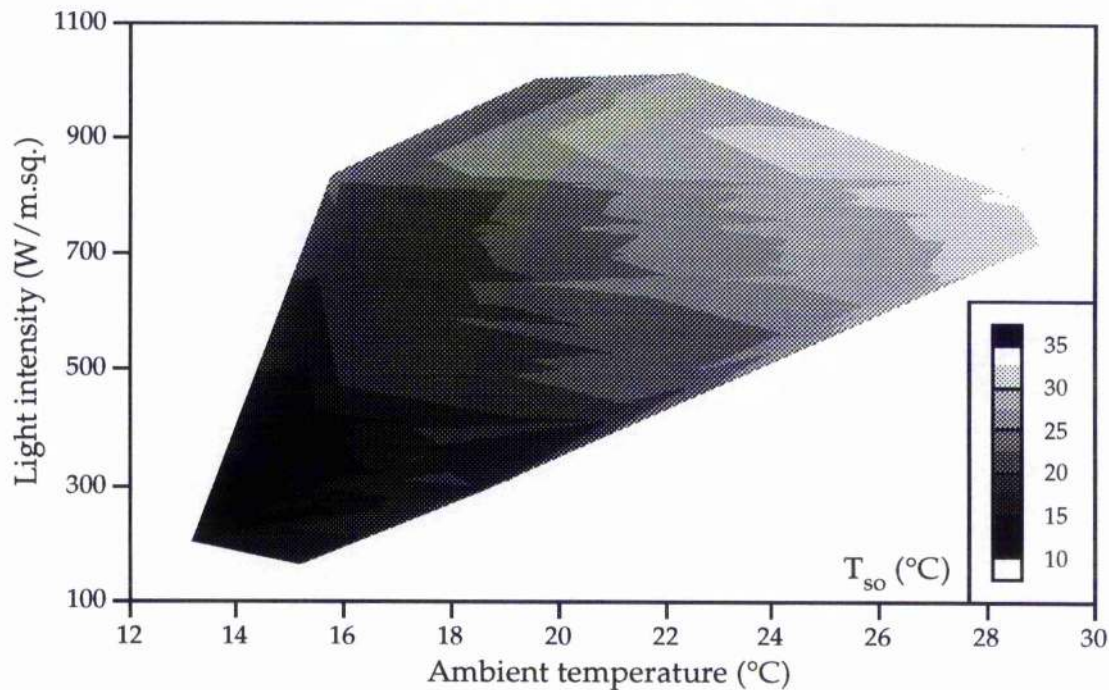


Fig. 4.11. (a) Ambient temperature (T_a) as a function of light intensity and time of day ($n = 49$). (b) Standard operative temperature (T_{so}) as a function of ambient temperature and light intensity ($n = 49$). Data collected on 7 days through the season of 1992.

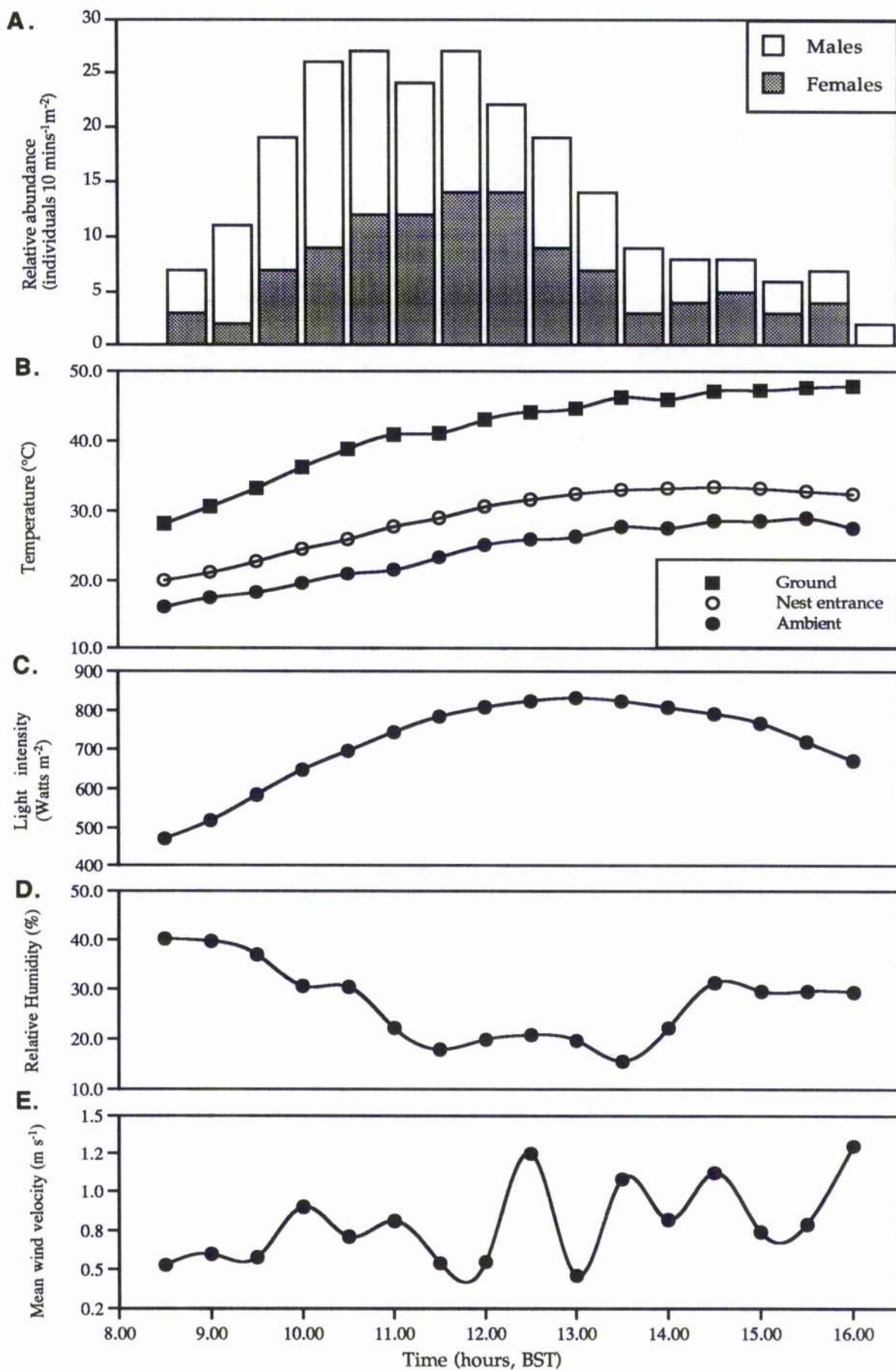


Fig. 4.12. Abundance and microclimate changes over time at Invergowrie on 14.8.92. (a) Relative abundance of female and male *H. rubicundus*. (b) Ambient, ground and nest entrance temperatures. (c) Light intensity. (d) Relative humidity. (e) Mean wind velocity.

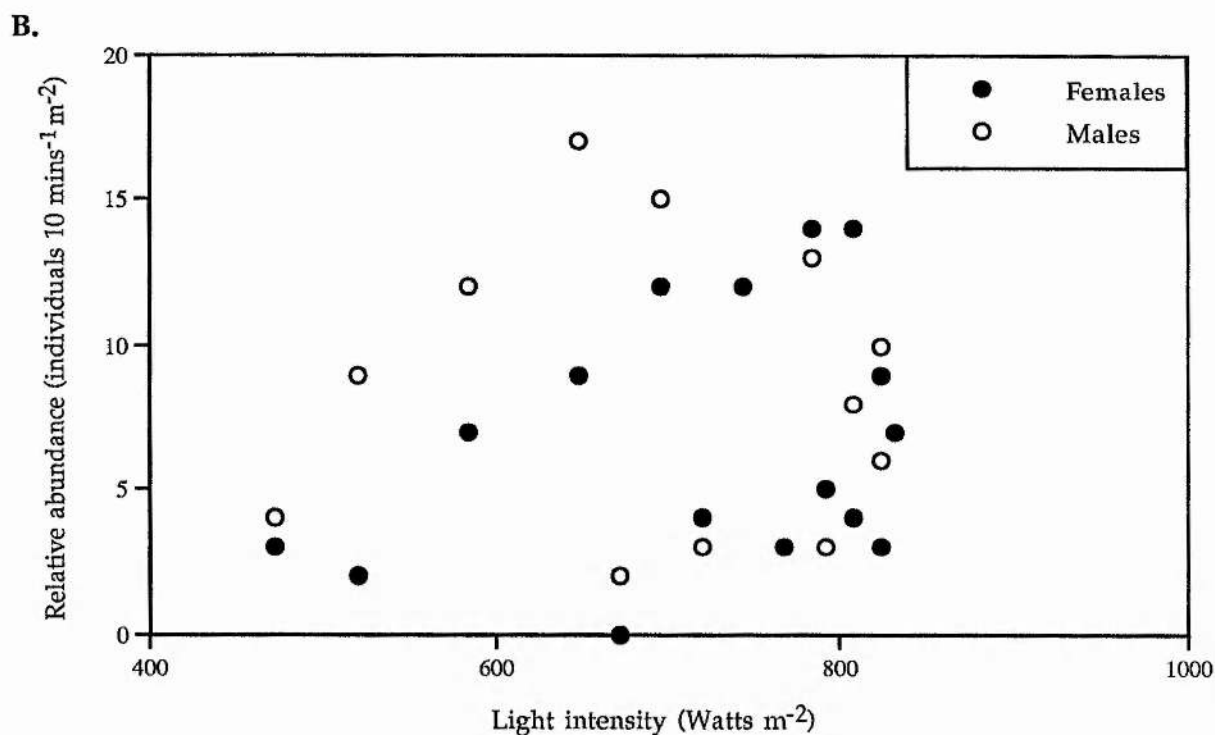
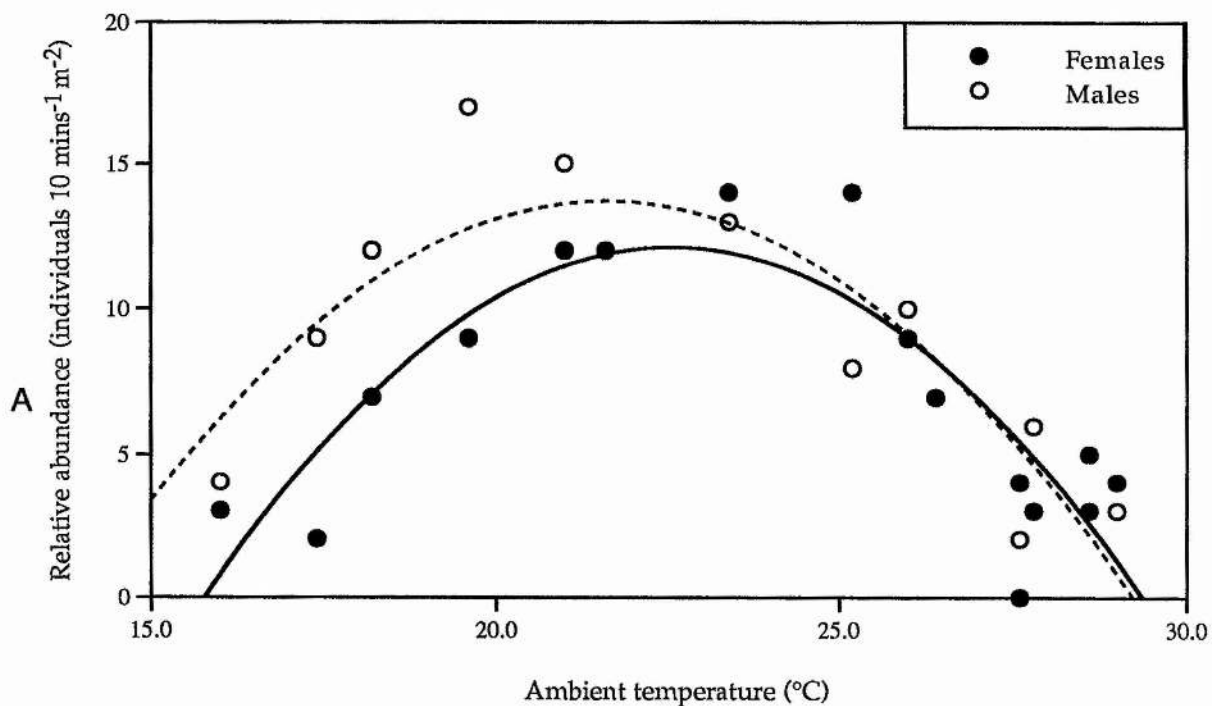
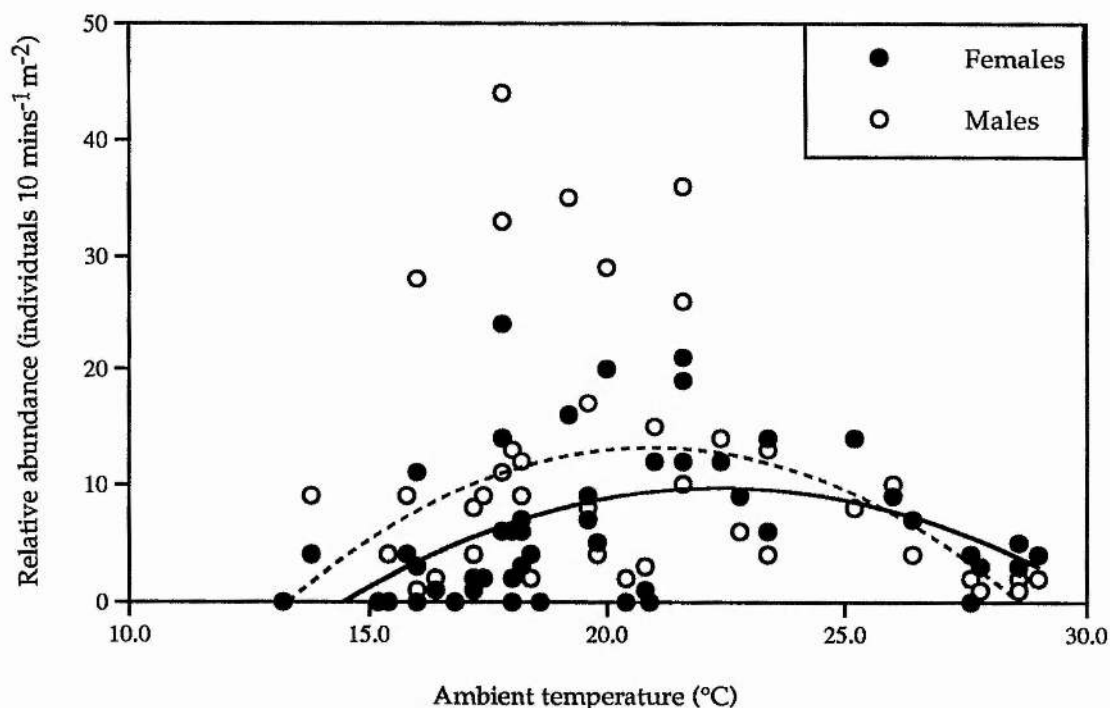


Fig. 4.13. Relative abundance of female and male *H. rubicundus* at Invergowrie on 14.8.92. **a)** Across a range of ambient temperatures: females ($y = -0.262x^2 + 11.835x - 121.422$, $r^2 = 0.717$, d.f. = 13); and males ($y = -0.237x^2 + 10.240x - 96.948$, $r^2 = 0.827$, d.f. = 13); neither curve deviated significantly ($p = 0.100$ and 0.916 respectively) from a second order polynomial model. **b)** Across a range of light intensities: Neither a 1st or 2nd order polynomial model fitted these data for either males or females (refer to section 4.4.2).

A.



B.

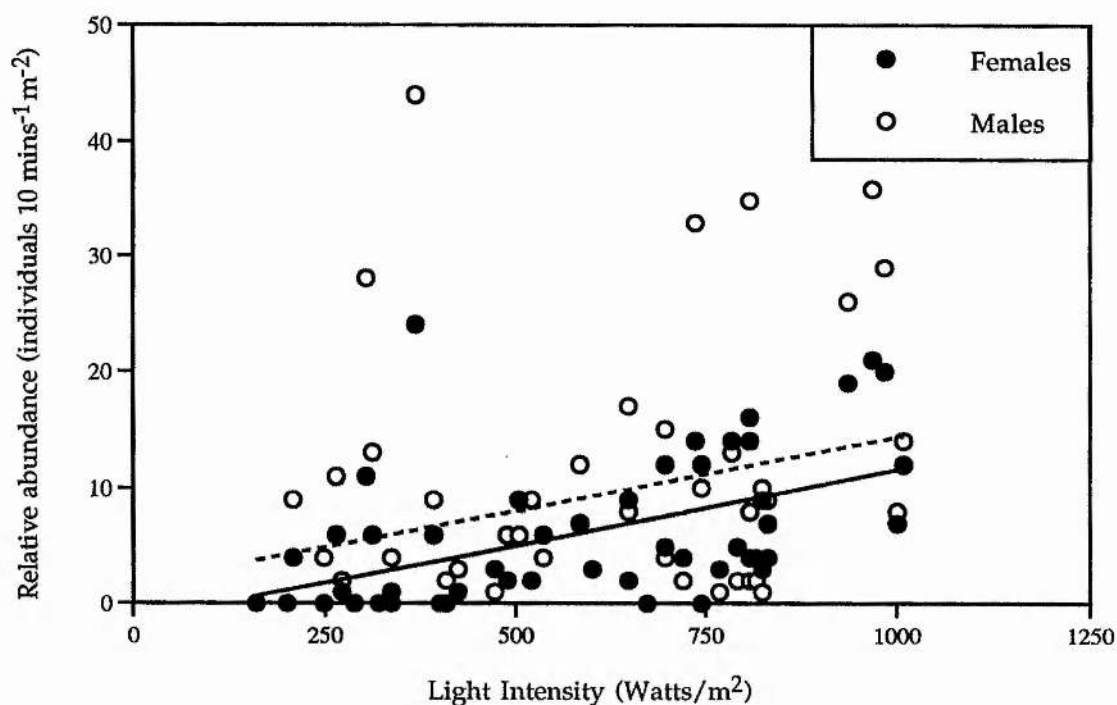
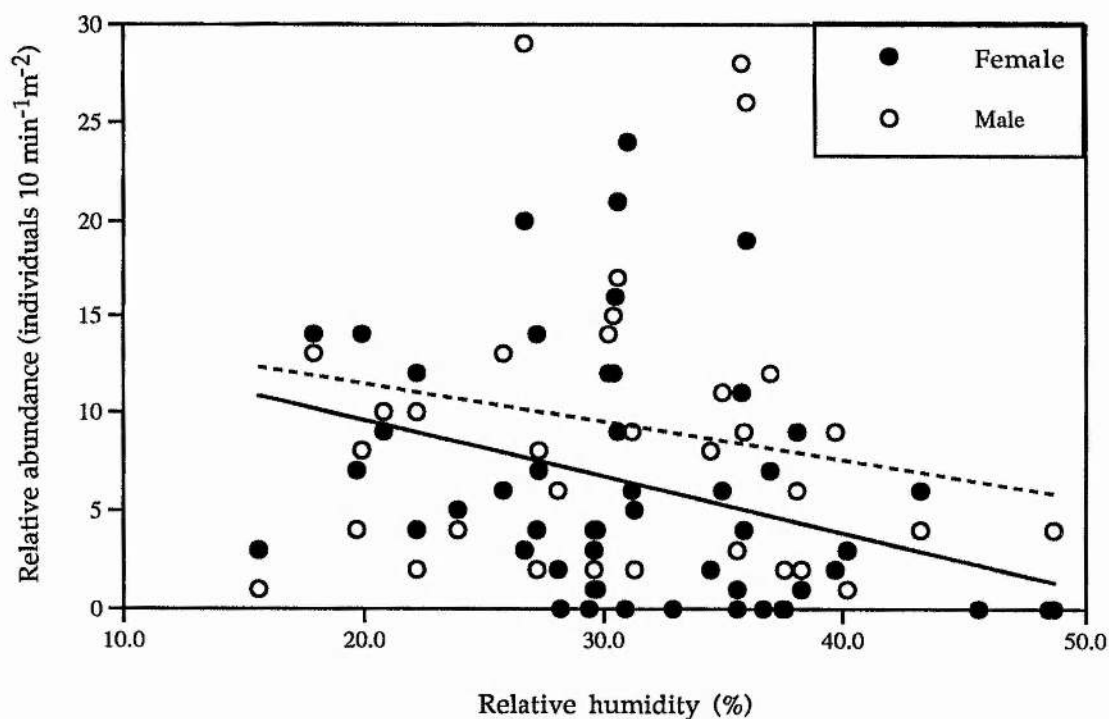


Fig. 4.14. Variation in relative abundance of *H. rubicundus* with: (a) Ambient temperature (female: $y = -0.154x^2 + 6.899x - 67.600$, $r^2 = 0.224$, d.f. = 47 and male: $y = -0.225x^2 + 9.412x - 85.428$, $r^2 = 0.146$, d.f. = 47); neither curve deviated significantly ($p = 0.129$ and $p = 0.051$ respectively) from a second order polynomial model; (b) Light intensity (females: $y = 0.013x - 1.426$, $r^2 = 0.255$, d.f. = 48, $p < 0.001$ and males: $y = 0.012x + 1.824$, $r^2 = 0.078$, d.f. = 48, $p = 0.024$). Data for combined observations made throughout the season of 1992 (totalling 7 days).

A.



B.

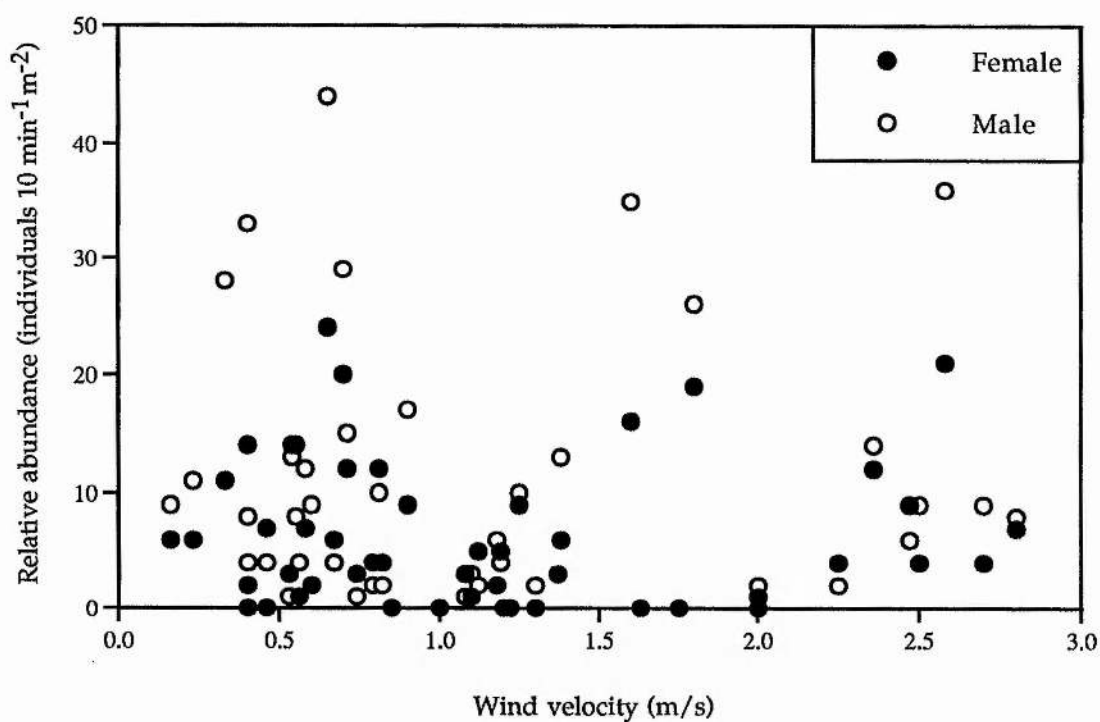
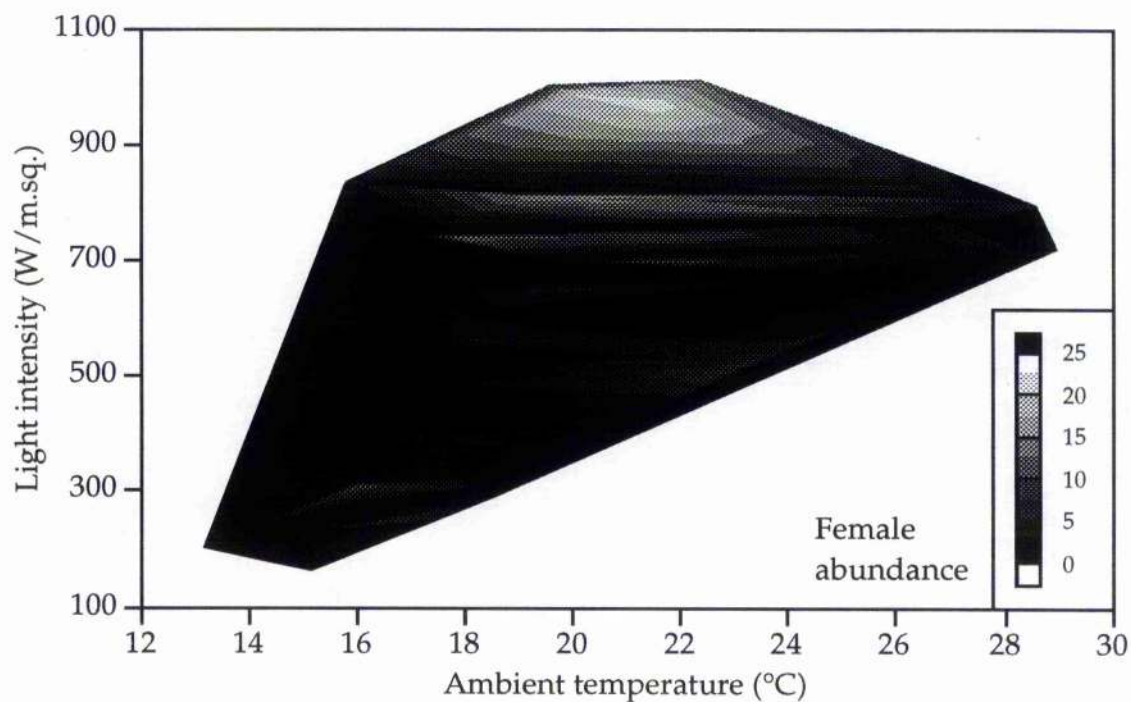


Fig. 4.15. Relative abundances of individuals as a function of: (a) Relative humidity (females: $y = -0.286x + 15.289$, $r^2 = 0.111$, $p = 0.006$ and males: $y = -0.196x + 15.379$, $r^2 = 0.017$, $p = 0.090$) and (b) Wind velocity ($p > 0.5$ for both).

A.



B.

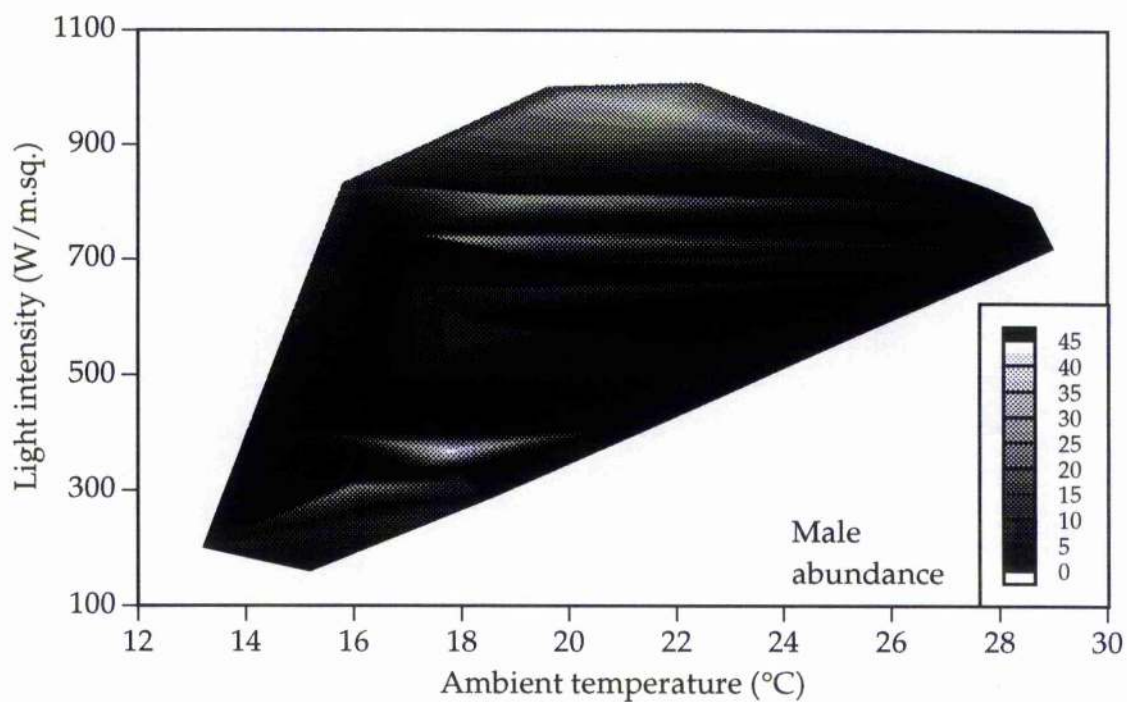


Fig 4.16. Relative abundance (individuals flying/10 mins/ m^2) as a function of ambient temperature and light intensity ($n = 49$) for: (a) Females and (b) Males.

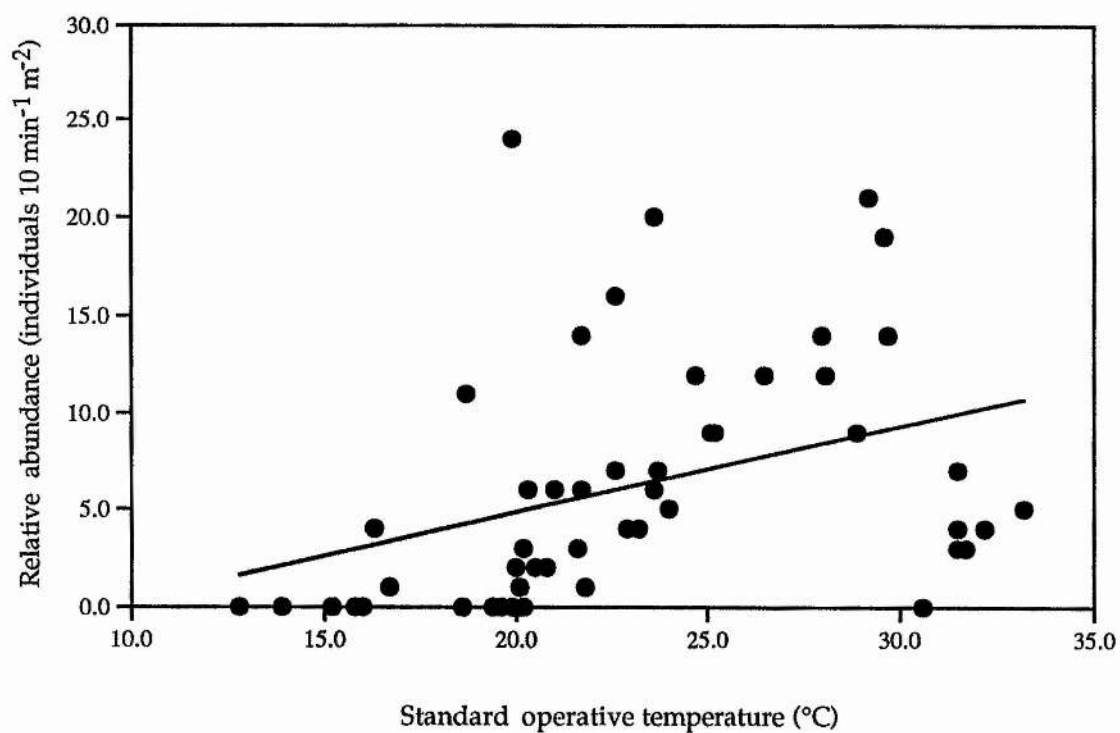
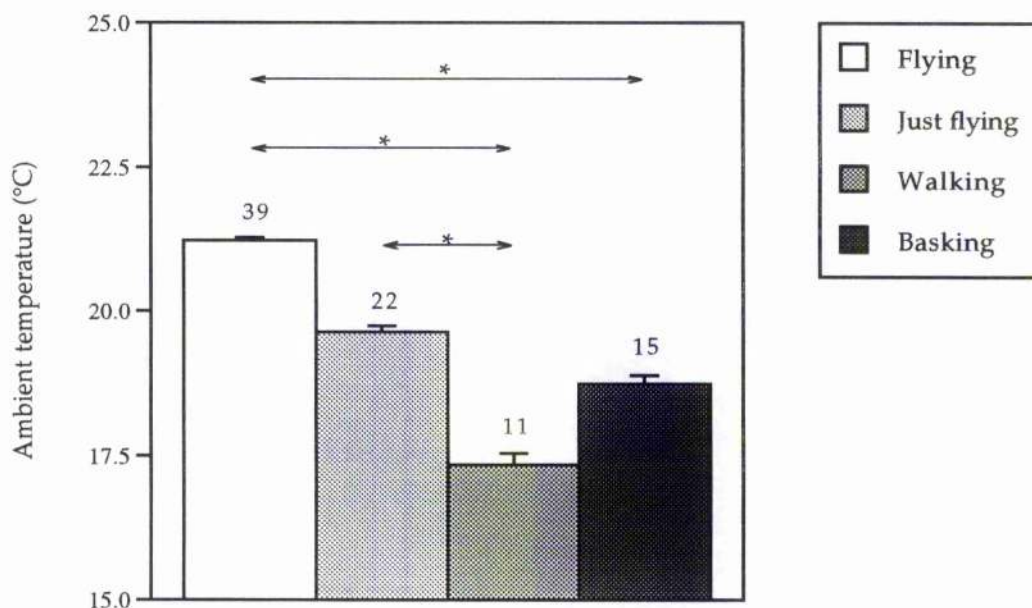


Fig. 4.17. Relative abundance of females as a function of standard operative temperature ($y = 0.447x - 4.090$, $r^2 = 0.149$, d.f. = 47, $p = 0.001$).

A.



B.

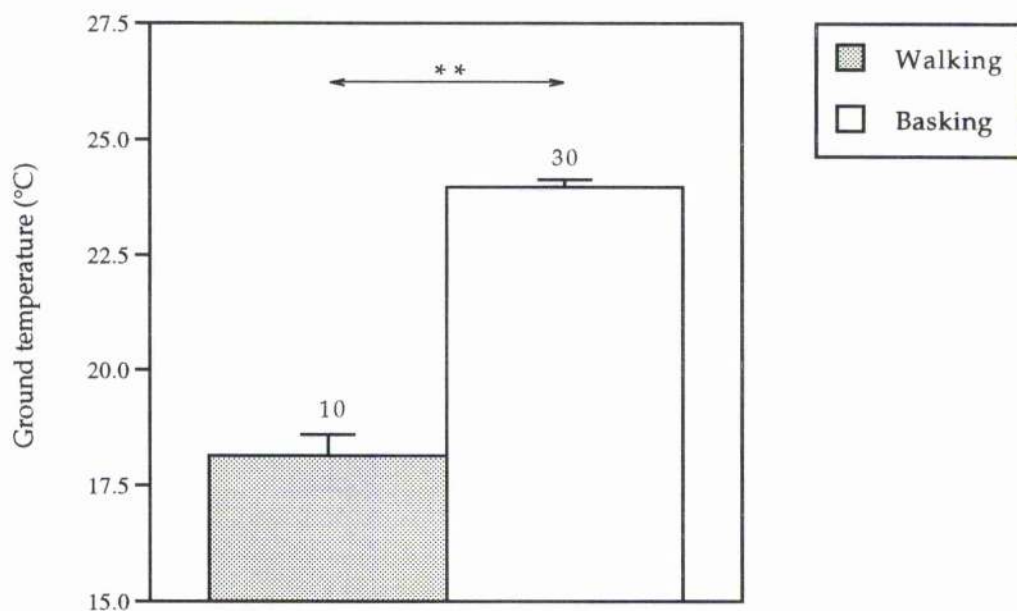
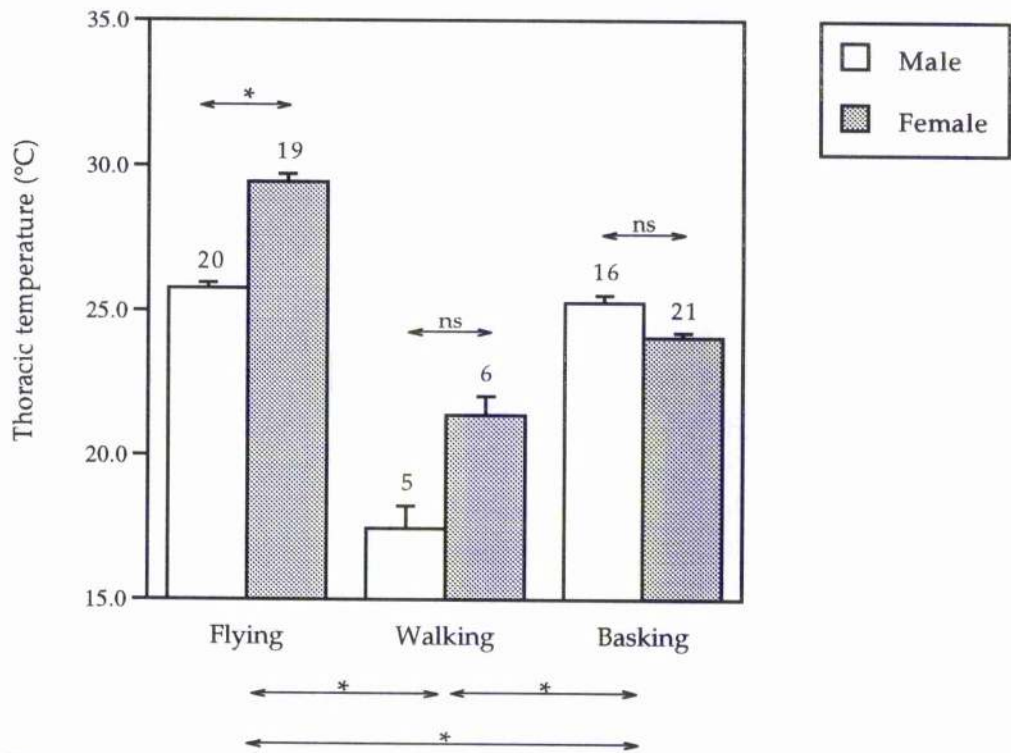


Fig. 4.18. (a) Mean ambient temperatures for the four activities recorded. **(b)** Mean ground temperatures for basking and walking individuals. Both graphs use data grouped for sex. '*' indicates the difference in means is significant ($p < 0.05$); '**' indicates the difference is highly significant ($p < 0.01$); all other differences are not significant. Numbers above columns give sample sizes.

A.



B.

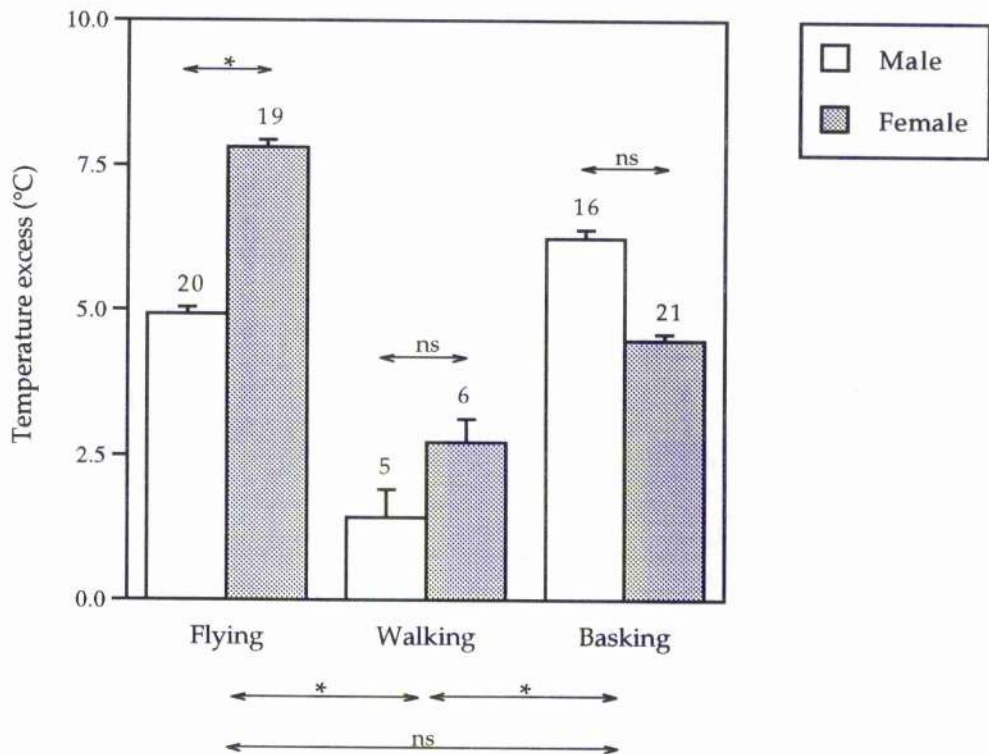
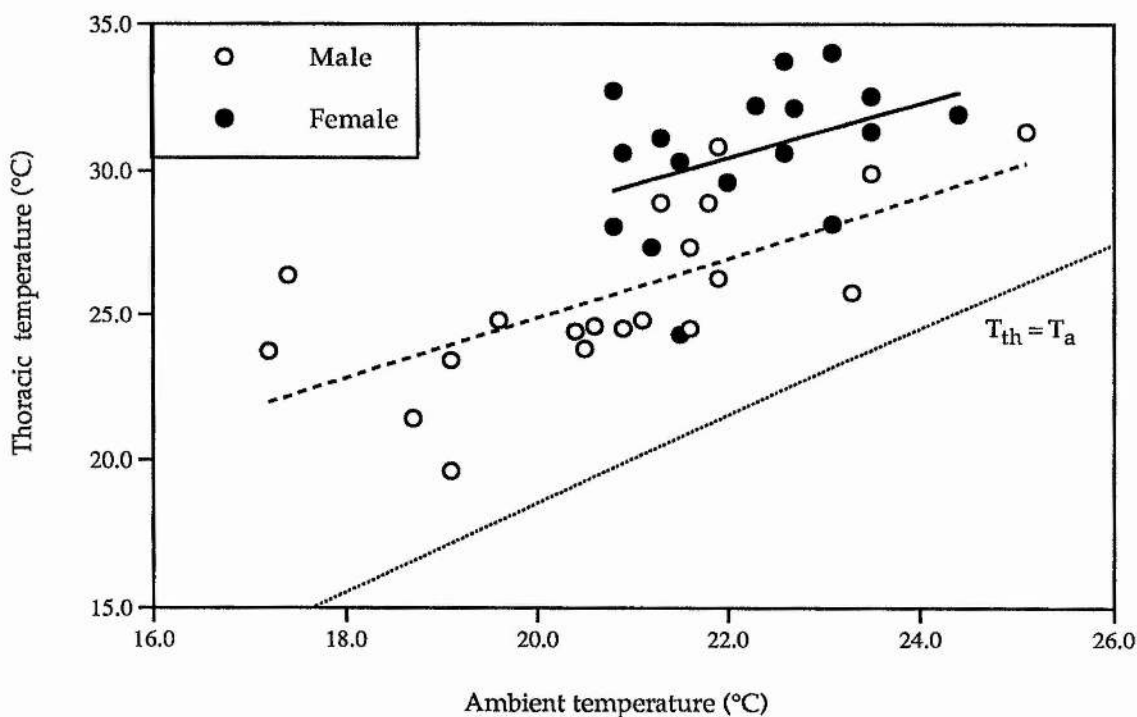


Fig. 4.19. (a) Mean thoracic temperatures of males and females for three activities. (b) Mean temperature excesses of males and females for three activities. '*' indicates the difference is significant ($p < 0.05$); 'ns' indicates the difference is not significant. Numbers above columns indicate the sample sizes.

A.



B.

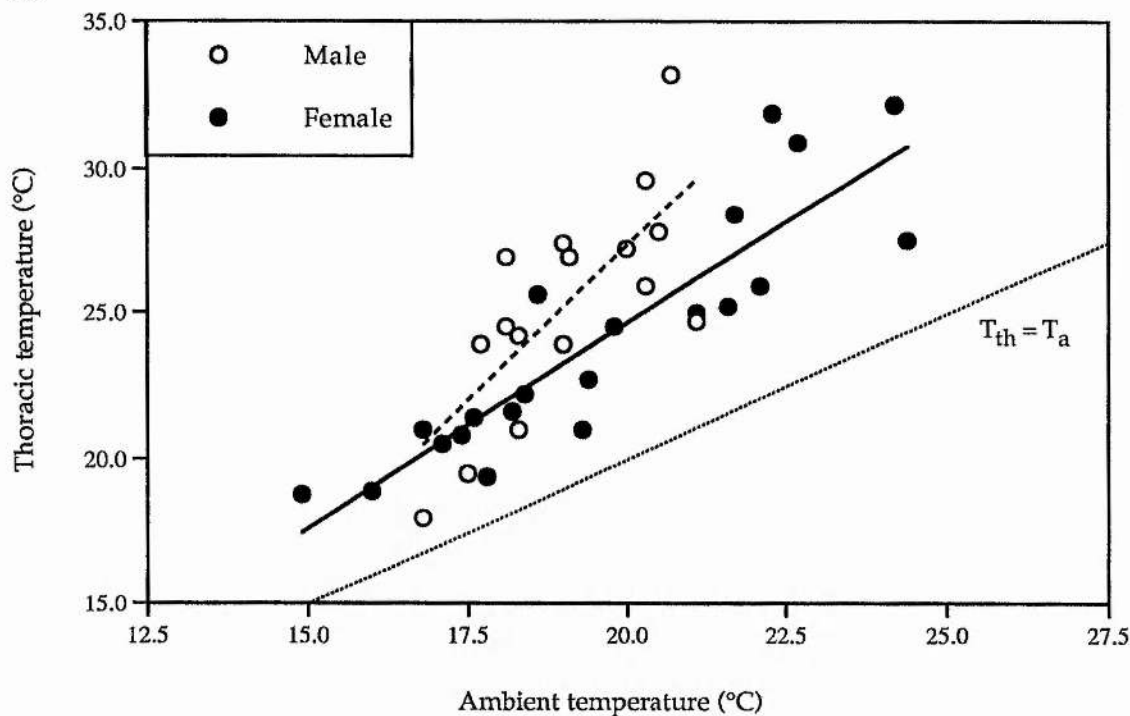
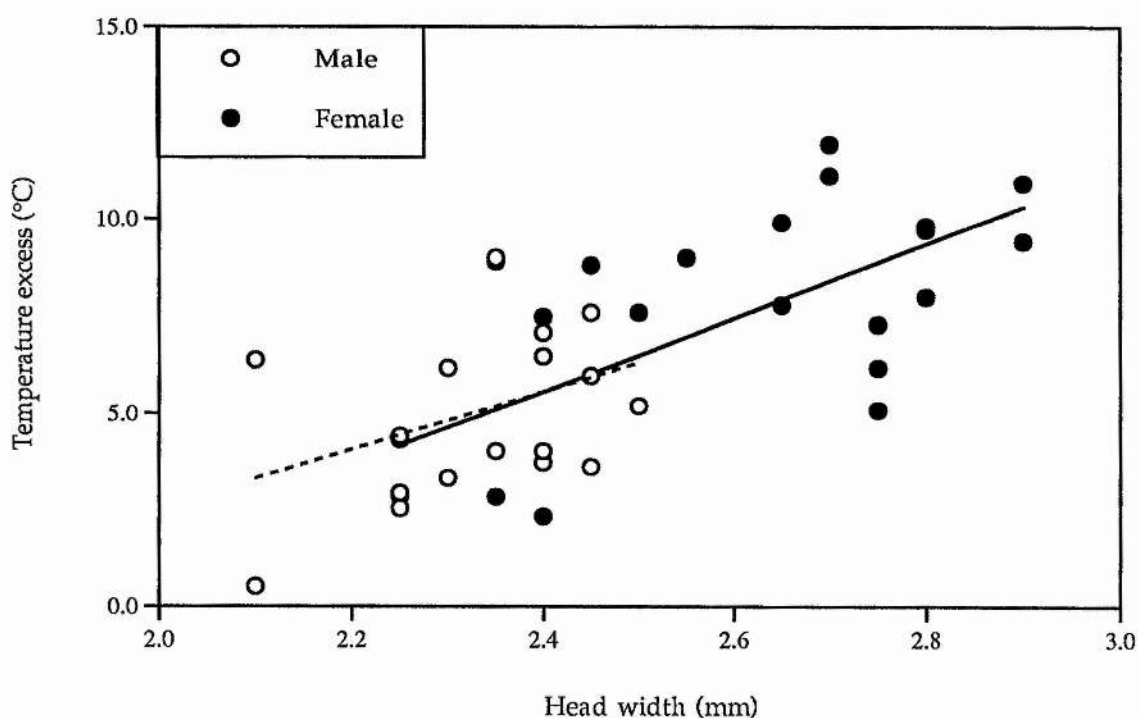


Fig 4.20. Thoracic temperature as a function of ambient temperature for: (a) Flying individuals (males: $y = 1.051x + 3.872$, $r^2 = 0.463$, d.f. = 18, $p = 0.001$ and females: $y = 0.924x + 10.089$, $r^2 = 0.162$, d.f. = 15, $p = 0.110$). (b) Basking individuals (males: $y = 2.136x - 15.405$, $r^2 = 0.544$, d.f. = 14, $p = 0.001$ and females: $y = 1.396x - 3.289$, $r^2 = 0.802$, d.f. = 19, $p = 0.001$).

A.



B.

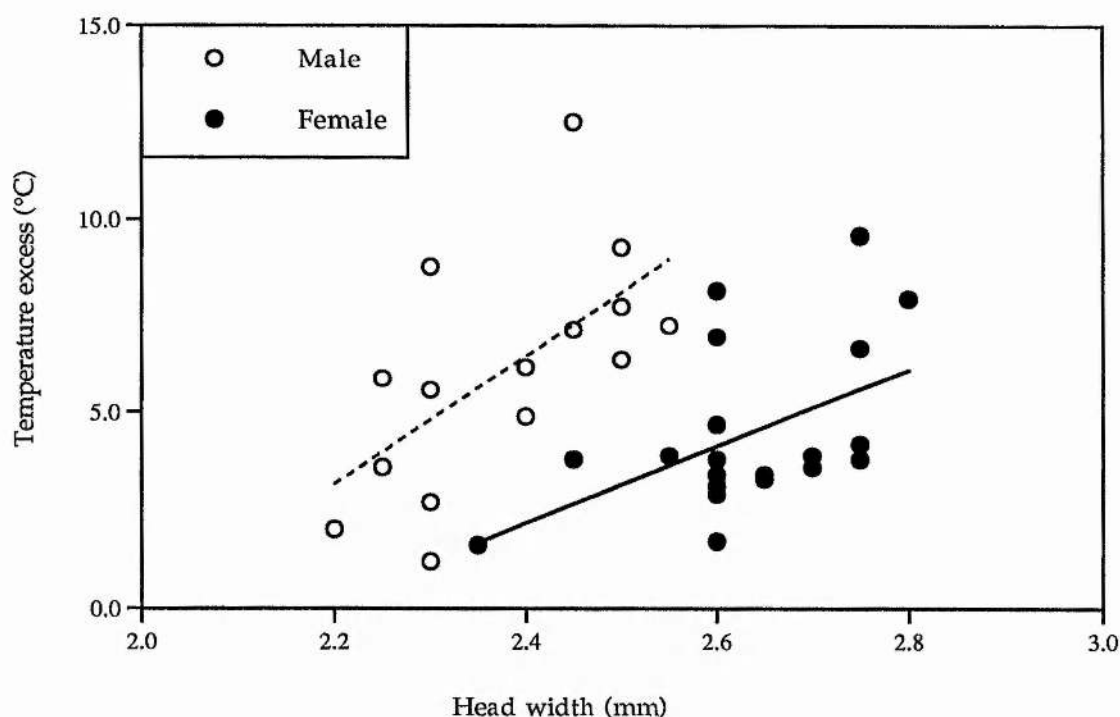
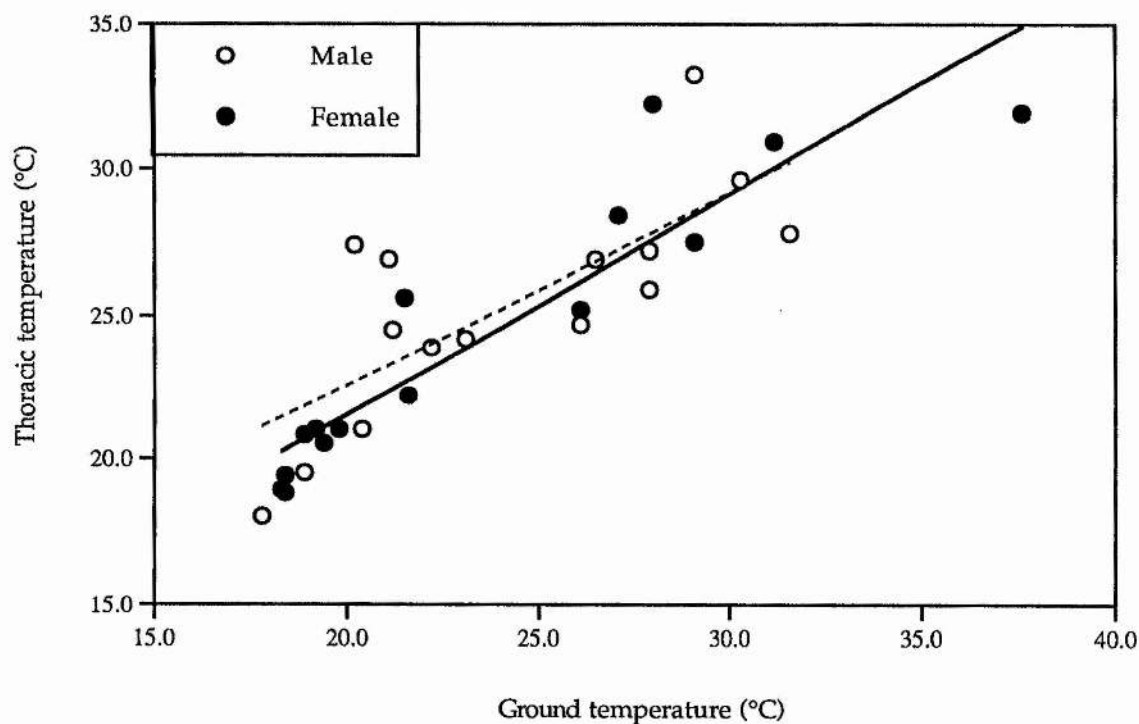


Fig. 4.21. Temperature excess as a function of head width for: (a) Flying individuals (males: $y = 7.632x - 12.737$, $r^2 = 0.147$, d.f. = 17, $p = 0.105$ and females: $y = 9.452x - 17.114$, $r^2 = 0.413$, d.f. = 17, $p = 0.003$). (b) Basking individuals (males: $y = 16.679x - 33.547$, $r^2 = 0.390$, d.f. = 13, $p = 0.003$ and females: $y = 9.932x - 21.679$, $r^2 = 0.238$, d.f. = 19, $p = 0.025$).

A.



B.

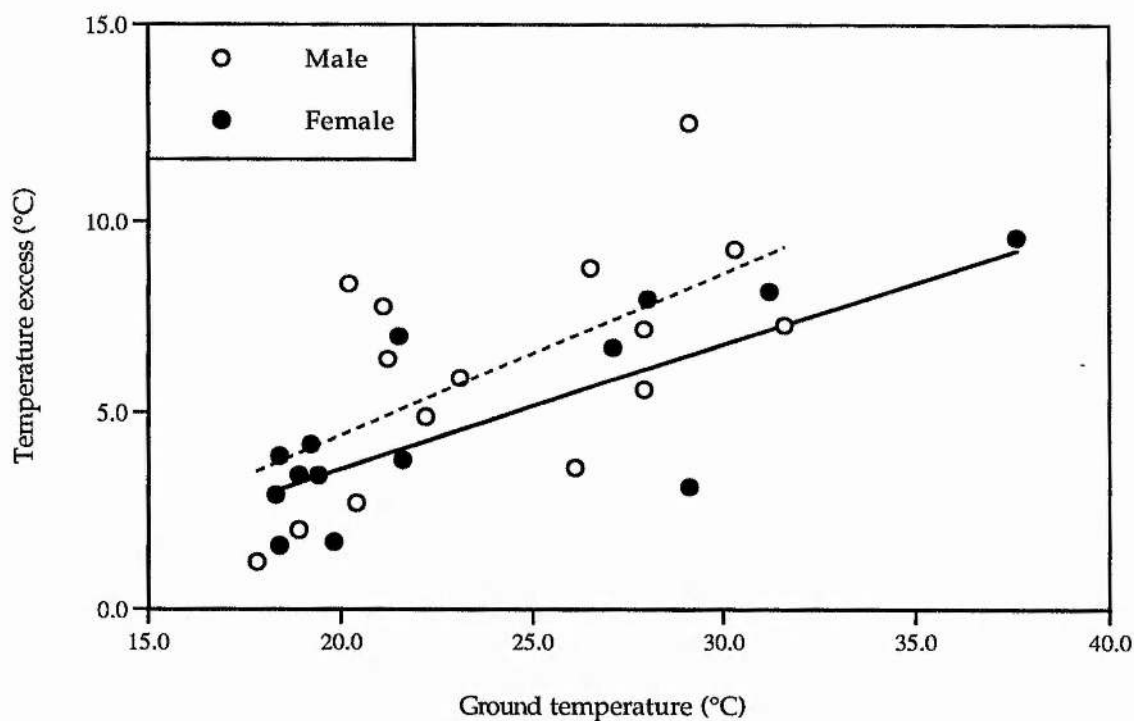
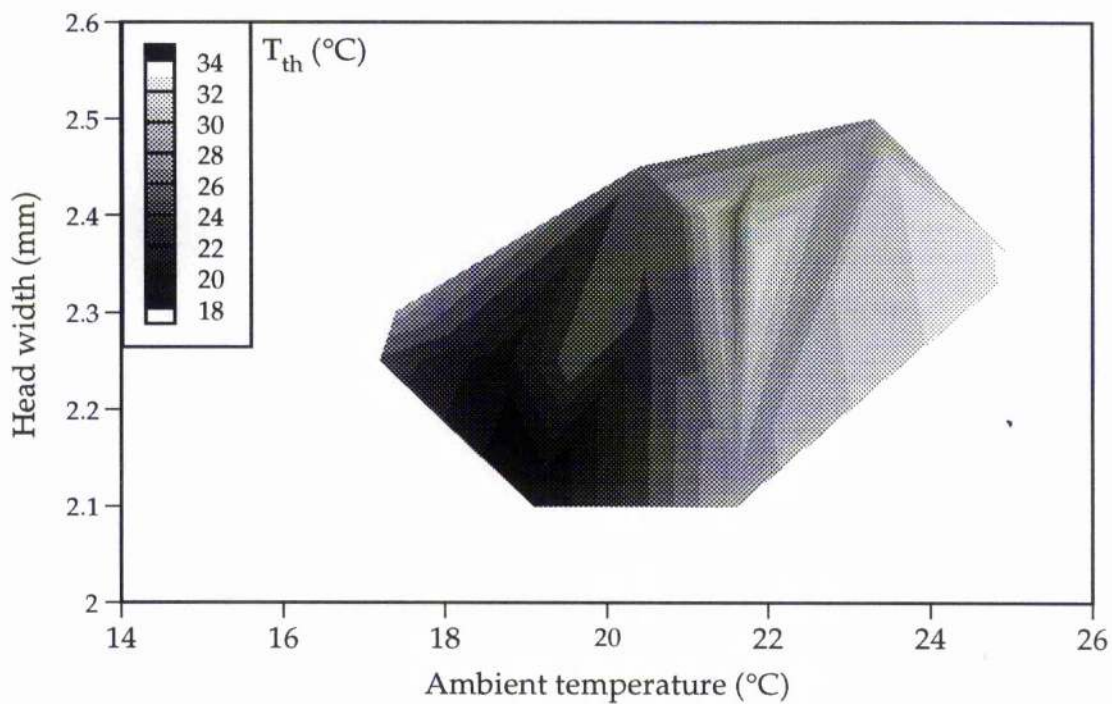


Fig. 4.22. (a) Thoracic temperature as a function of ground temperature for basking individuals (males: $y = 0.760x + 6.328$, $r^2 = 0.851$, d.f. = 13, $p = 0.000$ and females: $y = 0.661x + 9.335$, $r^2 = 0.572$, d.f. = 13, $p = 0.001$). (b) Temperature excess as a function of ground temperature for basking individuals (males: $y = 0.423x - 4.033$, $r^2 = 0.381$, d.f. = 13, $p = 0.014$ and females: $y = 0.323x - 2.90$, $r^2 = 0.583$, d.f. = 13, $p = 0.001$).

A.



B.

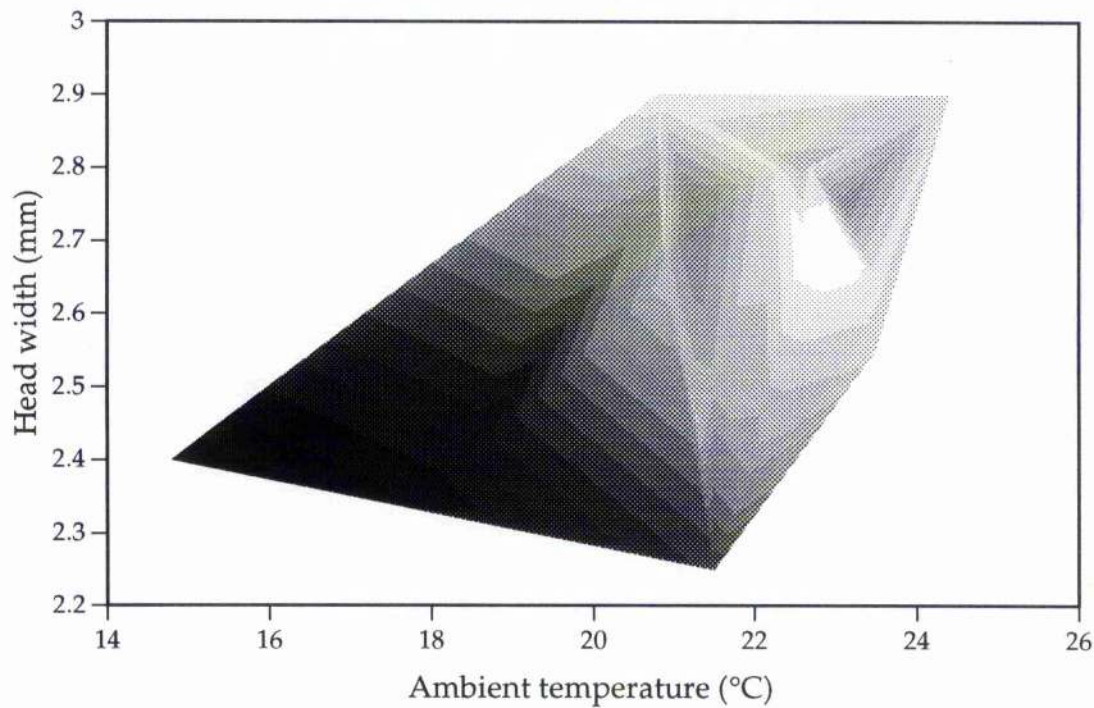
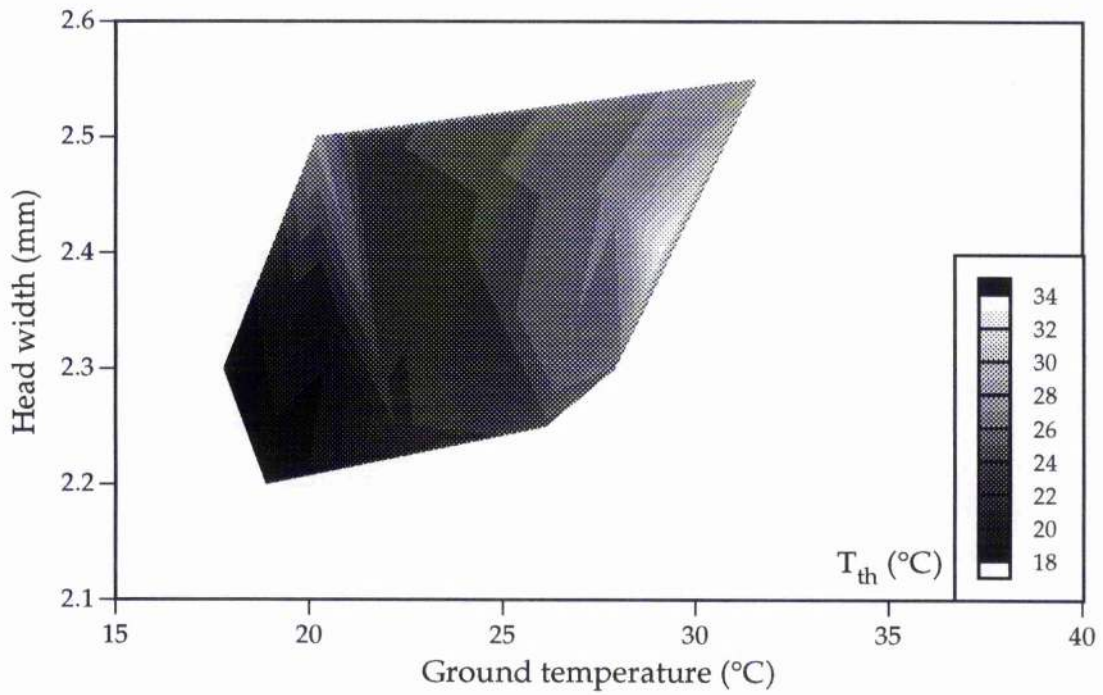


Fig. 4.23. Contour graphs showing thoracic temperature (T_{th}) as a function of ambient temperature and head width for flying individuals: (a) Males (n = 20) and (b) Females (n = 19).

A.



B.

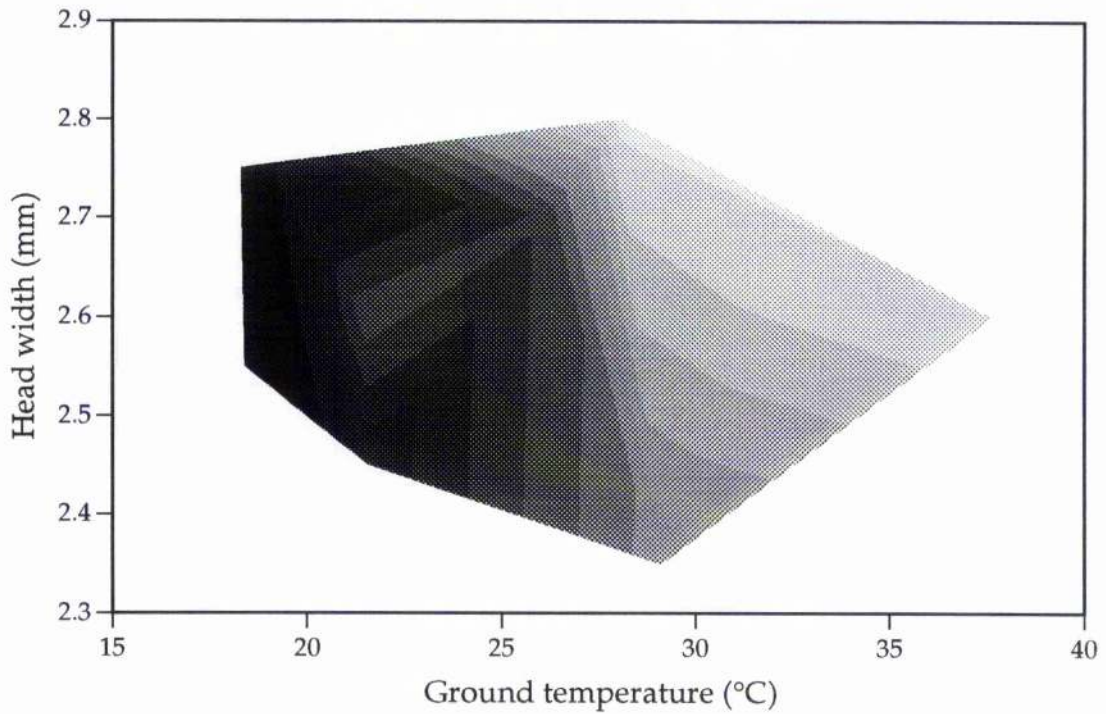
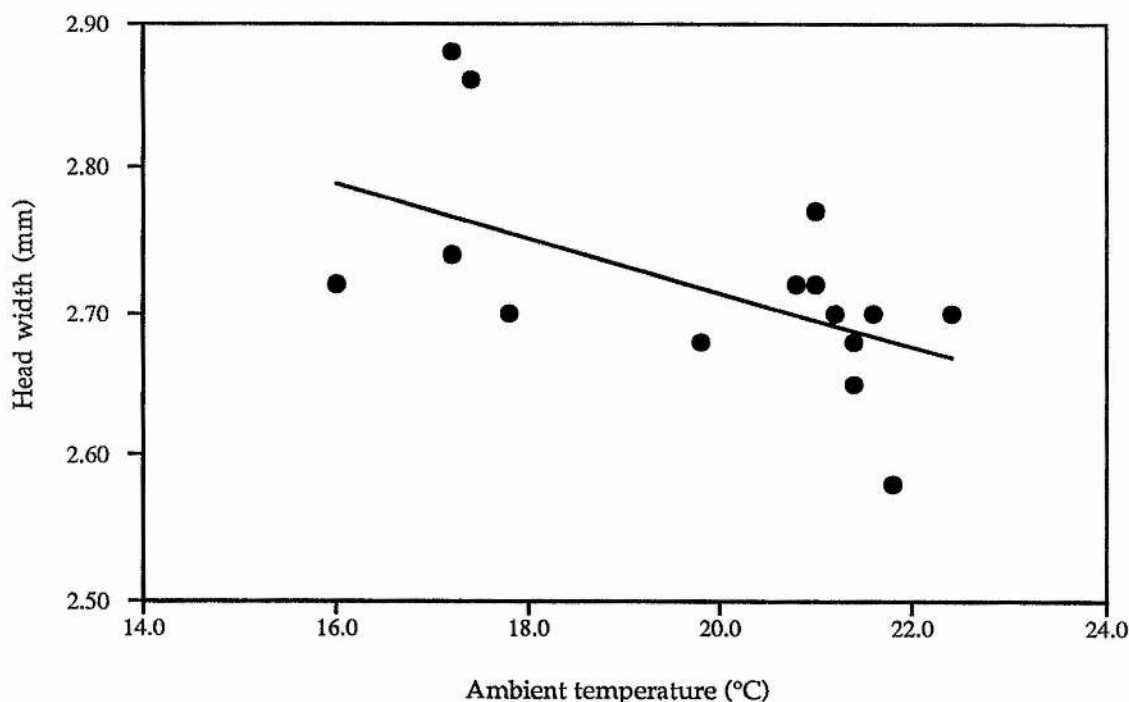


Fig. 4.24. Contour graphs showing thoracic temperature (T_{th}) as a function of ground temperature and head width for basking individuals: (a) Males ($n = 16$) and (b) Females ($n = 21$).

A.



B.

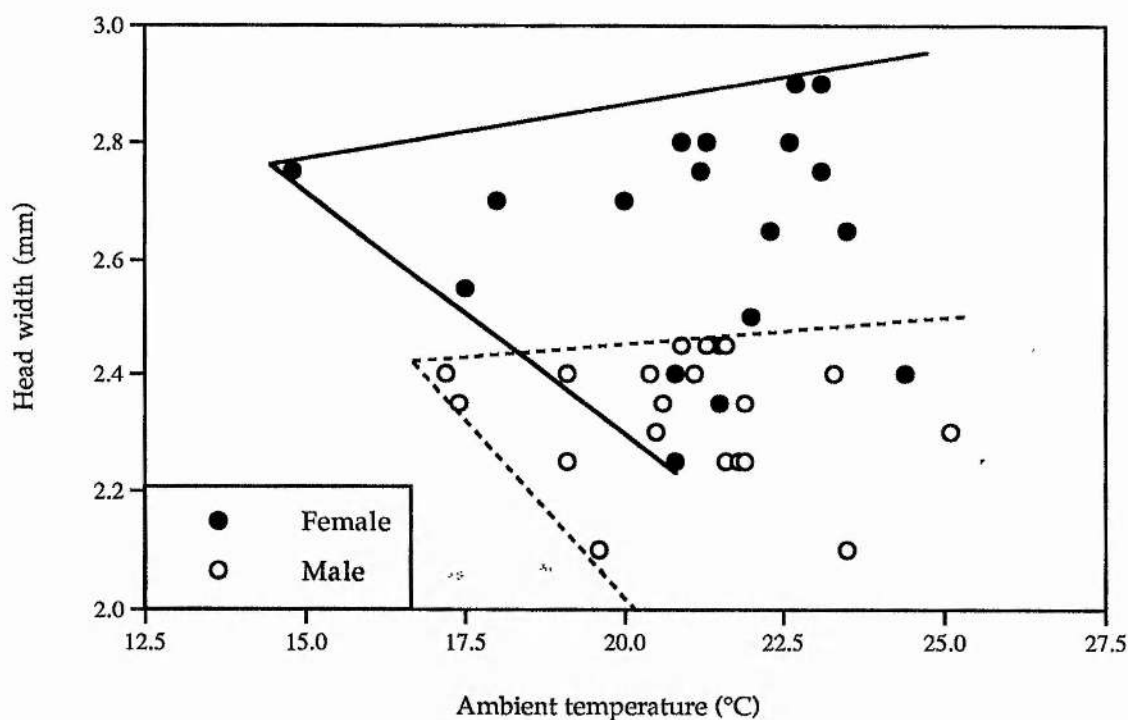
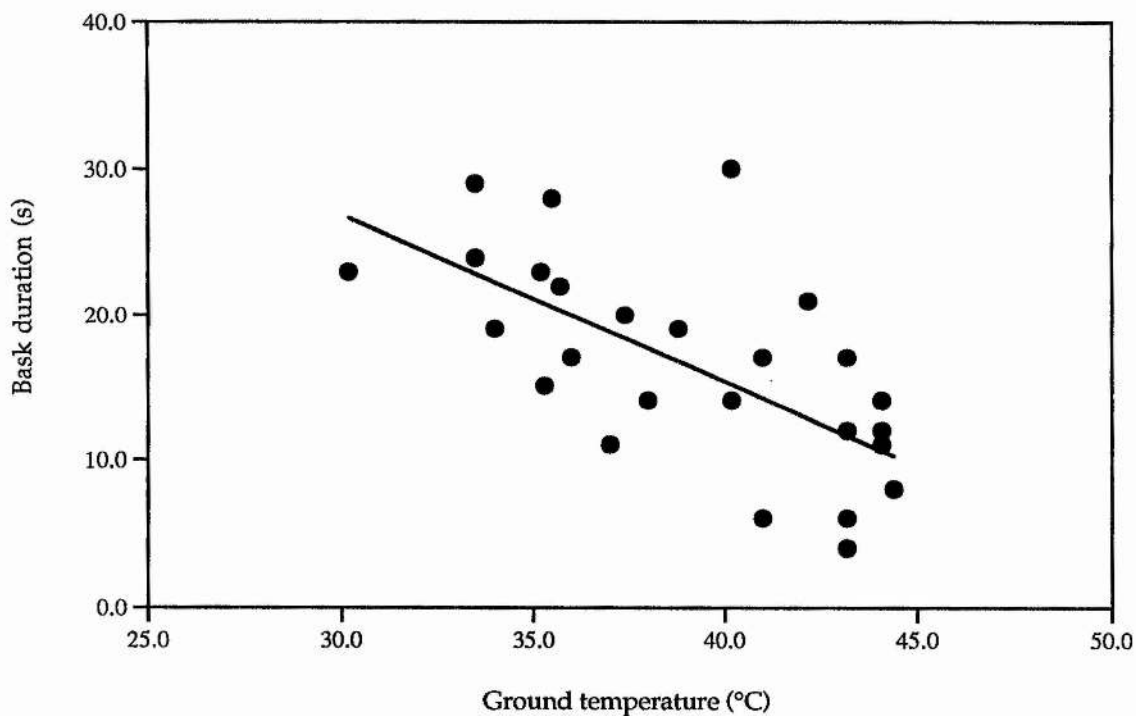


Fig. 4.25. (a) Female head width and the ambient temperature at which first emergence from the nest occurs ($y = -0.019x + 3.091$, d.f. = 13, $r^2 = 0.304$, $p = 0.027$). Recordings made on 10.6.94, 13.6.94 and 26.6.94. (b) Head width and ambient temperature for flying individuals. Solid and dashed lines show the estimated activity windows for females and males respectively (see text for explanation). Measurements made throughout the field season of 1992.

A.



B.

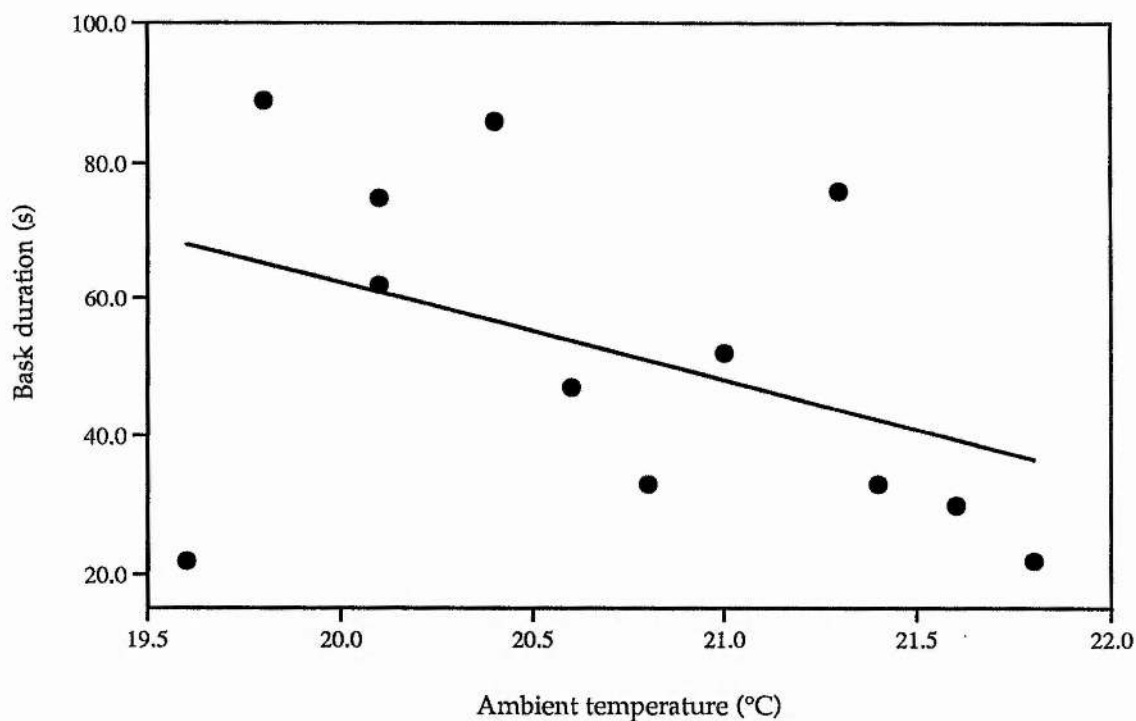


Fig. 4.26. (a) Bask duration as a function of ground temperature for females basking on the banking surface at Invergowrie ($y = -1.161x + 61.75$, $r^2 = 0.426$, d.f. = 25, $p < 0.001$). (b) Basking duration as a function of ambient temperature for females basking in their burrow entrances ($y = -14.361x + 349.64$, $r^2 = 0.177$, d.f. = 10, $p = 0.173$). Recordings made on 13.6.94, 28.6.94, 1.7.94 and 8.8.94.

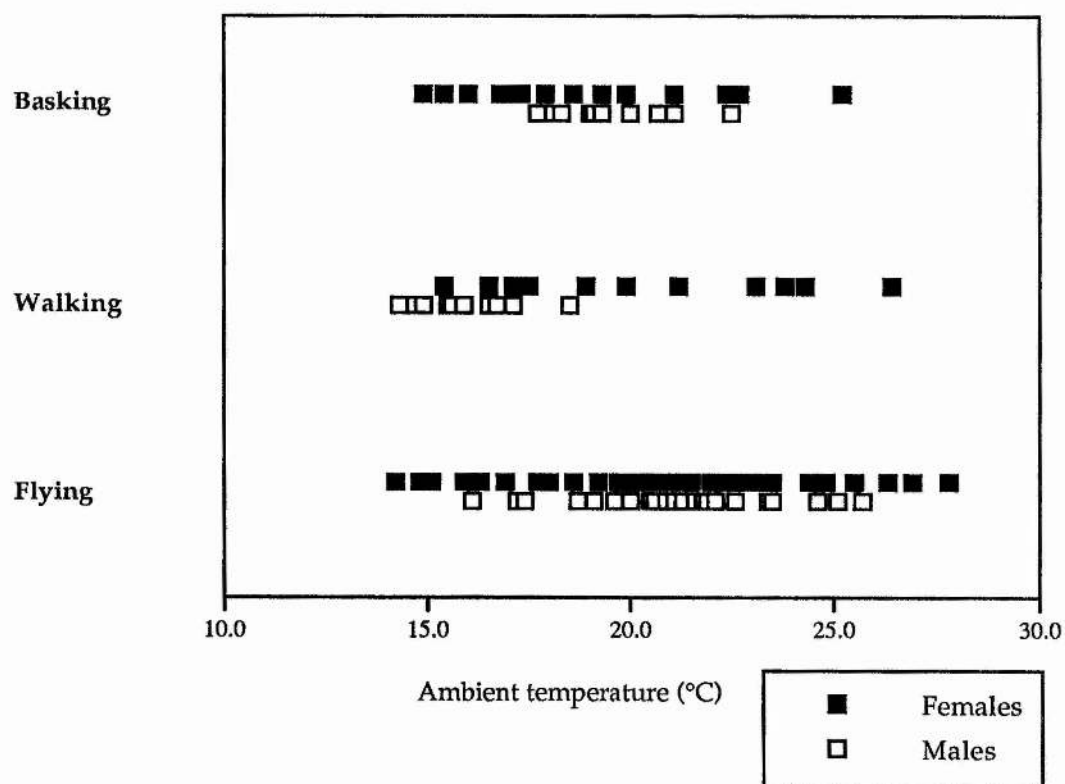


Fig. 4.27. Ambient temperatures at which males and females are observed displaying various activities. Recordings made on many days throughout the 1992 field season and under a variety of ambient conditions. The data set also includes data from other studies in this chapter.

Chapter 5 Nest-site selection

5.1 Introduction

5.1.1 Abiotic and biotic factors examined

Nest-site selection by *H. rubicundus* can be split into more than one level. At the very broadest scale certain basic requirements must be satisfied. These include a need for: a substrate that can be burrowed into; an absence of dense vegetation; exposure to several hours of direct sunlight each day; and an area free of water-logging. A complete list of prerequisites would be enormous, and examination of such broad characteristics is of little use when investigating nest-site selection in hypogeous Hymenoptera, since in a heterogeneous environment relatively little area will satisfy these basic requirements.

What is more interesting is the variation in suitability within a given patch of ground that meets the above criteria. Individual females then show differential preferences between points in close proximity to one another, and so a choice is exhibited. This is reflected in the relative densities within an aggregation, and analysis of this is the main aim of this study. In addition, at the individual nest level, the spatial patterning within an area also reveals other small scale factors that influence female preference.

There is a vast array of possible factors that could influence nest-site selection in ground nesting Hymenoptera. Most studies have examined only a very small number of these variables, and then attempted to use any findings to explain nest-site preferences (see chapter 1). There is no doubt that many of these factors are important in nest-site selection activity, but it is also very probable that other significant factors have been overlooked.

One study has, however, been much broader in its approach. Brockmann (1979) looked at a range of the biotic and abiotic factors which influenced nest-site preference in the wasp *Sphex ichneumoneus*. Several nesting requirements were identified, and significant correlations between nest density and some substrate properties were found. In addition nest-site fidelity was observed; thus high density aggregations were explained by both the utilisation of limited substrate and by philopatric behaviour. No other study has looked at a wide range of variables likely to influence nest-site selection in a fossorial bee species.

Cane (1991) investigated a few abiotic factors measured at the nest-sites of 32 bee species, but did not look at any relationship between these and the density of nesting. Certain substrate preferences were found, but these varied as much within a bee taxon than they did between taxa.

From the published work (reviewed in chapter 1), it has been possible to put together a comprehensive list of factors that have been found to alter the suitability of a nesting area for a wide range of bee and wasp species. The influences these have upon nesting density are given in Table 5.1, and the predictions made are explained in section 1.2.

These factors have then been examined, in detail, for the single bee species *H. rubicundus* in order to explain the high levels of density observed in the aggregations of this species; both within (Table 5.2) and across (Table 5.5) sites.

As the number of ecological factors that could explain variation in nest density is very large and so the type of analysis that is undertaken is important. A series of linear regressions, with density as the response variable, would be highly susceptible to type I errors, with this number of predictors being tested. The

Bonferroni technique could be applied to a table of multiple correlations, but it is often considered too stringent a modification to make and seldom used (Rice 1989). Instead multiple regression has been used wherever possible, as this controls for type I errors.

Many of the factors tested are likely to be closely related, especially when examining edaphic and microclimatic variables. A female bee may test various potential points to initiate a nest and so use cues from the environment to make the decision whether to dig or not. It is easy to measure a large number of properties of the environment, but it may be difficult to determine which are the ultimate and which are the proximate factors determining nest-site suitability. For instance the maintenance of a warm nest is selectively advantageous for both adult and offspring (ultimate factor); and a moderately angled banking, free of vegetation, with a southern aspect are all proximal factors which help ensure that a nest's temperature is kept elevated. It may then not be necessary for a searching female to perceive the ultimate factor determining nest-site suitability, but instead use one or more proximate factors that are closely correlated to it.

The various factors were tested by using areas of varying nest density within a site (Invergowrie), and areas of maximum nest density across sites in the UK. It is assumed that an area of maximum density is the most locally favoured nesting site within a heterogeneous environment. Maximum and mean nest density for a particular site are highly correlated (see section 5.3.5), and so both maximum and mean densities will vary similarly with respect to other variables.

5.1.2 Female searching and digging behaviour

When females emerge, after overwintering in their hibernacula, they begin to search for a site to initiate a new nest. Initially they fly just above the banking

surface and hover around various surface features before moving on. The entrances of last seasons nests tend to be filled in after the winter, though those still open are often investigated. A single female may fly and hover for several minutes before choosing a point to land. This aerial search probably serves two functions: firstly to use visual clues of the banking to locate the general area of the natal nest from which the female emerged the previous season (see section 5.2.6); and secondly to allow the female to compare some coarse grain characteristics of the banking, such as slope angle and vegetation cover.

Once a female has landed she will walk over a small area of a few cm² several times and then start biting at a particular point with her mandibles. The biting usually lasts for a couple of seconds (mean = 2.21 ± 0.26 s (9)) before the female walks away. She may then quickly return and continue biting at the same point; or move off and investigate another point before returning to the original one. This cycle may be repeated many times (5.67 ± 1.86 (6)). A shallow exploratory dig is then initiated at one of the points of biting, and this can be anything from a small scrape to a several mm deep hole (17.64 ± 5.32 mm (11)). More than one test dig may be started by a female (1.57 ± 0.30 (11)); and if an old tunnel is being investigated then a test excavation may be started here.

Finally a female will settle on a single test digging point and continue to dig without interruption (except for short absences, presumably feeding trips); with the other test digs being abandoned. During this process females tend to avoid each other, although they will repeatedly examine abandoned test digs of other females. At any point in the above sequence a female may totally abandon her search and fly away to start searching elsewhere.

In addition to the walking and biting behaviour there are periods of apparent basking (mean = 18.46 ± 1.93 s (13)). Whether this is for thermoregulatory

purposes only (section 4.5), or in some way used to assess the thermal properties of the substrate, it is impossible to tell. Throughout the sequence of searching the female will often tap the substrate with her antennae; the exact purpose of this is not clear, but it is probable that it is used in some manner to aid selection of a suitable site for nest founding.

Through all of the above behaviour a female is obviously able to perceive certain physical parameters of the substrate and microclimate and then use these to decide on its suitability. The continual switching from one point of biting or digging to another probably allows the female to compare certain parameters of the substrate directly for various potential points of nest initiation. The final decision to found a nest thus only comes after a reasonably involved set of investigations have taken place. The sequence and number of repeats of the above behaviours displayed varies greatly between females.

5.2 Nest-site selection within a site (Invergowrie)

The factors in Table 5.2 fall into two discreet categories: macro factors (hardness, slope, surface temperature, soil temperature, soil humidity, pH and inorganic salt content) which were measured in 42 quadrats and micro factors (% gravel, % sand, % silt + clay, % organics and stone coverage) which were measured in 13 or 21 quadrats. As these two groups of factors have very different degrees of freedom it is sensible to carry out two separate multiple regressions to analyse their relationship with density. The results for the macro and micro factors are given in Table 5.3 and are considered the most valid in explaining variation in nest density. In addition, the linear regression for each individual factor is given in Table 5.2, these however, are treated with caution because of the problems outlined in section 5.1.1.

5.2.1 Soil hardness

For most ground nesting Hymenoptera the excavation of a nest represents a considerable investment, both in terms of time and energy (Michener & Rettenmeyer 1956; Brockmann 1980). One would therefore expect that, within a limited area of substrate, females would choose to nest in positions where the cost of excavation was relatively low (providing other factors remain constant). At Invergowrie this is the case (Figure 5.1a), with the quadrats of highest nest density having the lowest unconfined compression strengths ($y = -0.037x + 2.252$, $r^2 = 0.339$, d.f. = 40, $p < 0.001$). The importance of hardness is confirmed from the multiple regression results in Table 5.3a ($p < 0.001$).

For the 42 quadrats measured the mean hardness was 1.66 ± 0.11 Kgf cm⁻², with a range of 0.58 to 3.17 Kgf cm⁻². In addition, examination of two areas close to the established aggregation, that initially appeared suitable for nesting but had no nests present, showed that both had hardnesses greater than 4.0 Kgf cm⁻² (Table 5.3). This is greater than for any quadrat containing nests; however other factors such as slope may also have accounted for the unsuitability of these two areas.

The maximum density recorded at Invergowrie, in 1994, was 37 nests m⁻², which is relatively low when compared to the other sites investigated. At this density the structural integrity of adjacent nests is easily maintained and there is little chance of neighbouring nests collapsing into one another. This is confirmed by an observed mean nearest neighbour distance (NND) of 68.3 ± 4.8 mm (37) and the fact that in all the nests excavated the posterior wall of a cell was never found to be more than 30 mm away from the main burrow.

5.2.2 Slope and aspect

At Invergowrie, aspect was the same for all the quadrats measured, and so cannot be of use in explaining any of the variation in nest density. The whole site does however face almost due south (168°); such an aspect helps maximise the amount of solar radiation that can be absorbed. The preference of south facing slopes (in the northern hemisphere) is well known in many ground nesting Hymenoptera (e.g. Rubink 1978; Weaving 1989). The different aspects recorded at other sites are discussed in section 5.3.2.

Nesting on sloping ground can have several advantages. These include increased insolation, if south facing, which will lead to greater warming of the substrate and in turn the nest; thus allowing the female to warm-up more quickly and attain a higher body temperature in the nest burrow. This is particularly useful earlier in the day and during cooler weather, with the result that females are able to dig and forage more effectively. A second benefit is the quicker development times of eggs and larvae. In addition to the thermal advantages of nesting on sloping ground, improved drainage through better surface run-off can be very important (Sakagami & Michener 1962), especially in areas that are prone to water logging.

The multiple regression results show that slope is an important factor in explaining variation in nest density (Table 5.3a: $p = 0.004$). *H. rubicundus* certainly seems to exploit these thermal advantages by nesting on sloping ground, and there is a definite preference for the steeper slopes which receive the greatest amount of insolation (Figure 5.1b: $y = 0.118x + 20.198$, $r^2 = 0.114$, d.f. = 40, $p = 0.024$). In addition, the two areas sampled with zero density had small slope angles (3.4° and 18.6° ; Table 5.4), however they also had unfavourable hardness values which could also have accounted for the absence of nests.

5.2.3 Soil temperature

A good indicator of the thermal properties of a particular piece of substrate is its surface temperature. A searching female may be able to determine whether a patch of ground is suitable, in thermal terms, by simply landing on its surface; females often spend many seconds basking (or in "basking-like behaviour") at various points on the banking. Whether through conduction or the absorption of long wave re-radiated heat, an individual would receive warmth from the substrate; this could well be used as a cue in determining the relative thermal suitability of a particular area. As one might expect females choose to nest preferentially in areas with warmer surface soils (Figure 5.2a: $y = 0.028x + 27.184$, $r^2 = 0.105$, d.f. = 40, $p = 0.021$). This was not, however, borne out in the multiple regression results ($p = 0.086$), and so should be considered as being of less importance than either hardness or slope. Bearing this in mind it can be proposed that in the areas of highest nest densities, females would need to spend the shortest times warming up in burrow entrances before being able to leave the nest (see section 4.5.4.E). A preference for the warmest substrate within a given area has been demonstrated for several fossorial Hymenoptera (Sakagami & Hayashida 1961; Rubink 1978; Brockmann 1979; Weaving 1989).

The surface temperature of a particular quadrat will depend upon many factors. Assuming that soil colour and particulate composition are not highly variable across the banking, then surface temperature should be largely a product of aspect and slope, since these two factors determine the relative amounts of solar radiation absorbed. It is therefore likely that a female is coincidentally selecting sites of a particular slope and aspect due to the favourable surface temperature characteristics that they generate.

The most relevant temperature measurement associated with egg and larvae developmental rates is the temperature at 50 mm, since this is the usual depth of nest cells. Soil temperature at this depth and surface temperature are closely related (Figure 5.2b: $y = 0.649x + 7.701$, $r^2 = 0.179$, d.f. = 40, $p = 0.005$). So in selecting a warm surface a female is ensuring a warm microhabitat for her offspring to develop in.

The importance of the effect of insolation on nest-site choice was clearly demonstrated by use of a shading experiment. Quadrat 34b was completely shaded by black gauze netting, throughout the day, for the whole of April and May 1994. In the previous year 22 nests had been founded in that quadrat and the following year, with the treatment, no new nests were founded. However the number of nests in the surrounding 12 quadrats remained unchanged between years (paired t-test: $T = 0.61$, $p = 0.55$).

5.2.4 Other abiotic factors

Several other abiotic factors that might be linked to nesting densities were investigated at Invergowrie. None of the soil particle fractions (gravel, sand, silt and clay) from the soil cores were found to be correlated with nest density (Table 5.2 & 5.3b). However all the samples typically had a combined silt and clay fraction of less than 5% of the total by weight; while the gravel and sand proportions each varied between 20% and 80% (Figure 5.3). This places all the soils in the sand/loamy sand/sandy loam category. Whether female *H. rubicundus* prefer this type of soil, or simply tolerate it for nesting purposes, is not obvious from the within site study. It does however indicate that soils with high proportions of fine particulate matter are probably not favoured (see section 5.3.3).

The amount of organic matter present, the pH, and the relative amounts of inorganic salts present all vary independently of density (Table 5.2 & 5.3b). Soil humidity, which would be expected to be a crucial factor in determining the suitability of a nesting substrate, is also uncorrelated with nest density. However, in order to minimise water loss from cells a soil with a high moisture content is desirable. Most bee cells have a relative humidity of nearly 100% (Roubik 1989), and with a mean soil humidity of $80.5 \pm 0.6\%$ (42) the difference between soil and cell is not great. This means that the hydrophobic cell lining of macrocyclic lactones (O'Toole & Raw 1991) is easily sufficient to maintain a high moisture level in the cell within a soil of this type.

Stone coverage and vegetation coverage had no significant influence on the observed density of nests. There was however a preference for the siting of nests adjacent to, or under, stones. In the quadrat of highest density, 57% ($21/37$) of all nests were initiated next to or under stones. A possible advantage of this is that nest entrances attain higher temperatures, due to the greater absorption of solar radiation by stones, and so result in quicker warm-up times. Nest entrance temperatures for nests associated with stones are significantly greater than those not associated with stones (Figure 4.9: two sample t test: $T = -2.36$, d.f. = 17, $p = 0.030$). However the selection of sites under stones may primarily be a response to parasite pressure and the elevated nest entrance temperature is merely a 'bonus'.

5.2.5 Parasitic Diptera

The relative abundances of *H. rubicundus* females and parasitic Diptera are closely related (Figure 5.4a: $y = 0.975x + 0.106$, $r^2 = 0.762$, d.f. = 70, $p < 0.001$). This is consistent with the mode of nest parasitism employed by the Diptera, whereby a flying host is followed to its nest. So nesting in an area of high density results in large numbers of parasitic Diptera being locally abundant.

The chance of a particular nest being parasitised does increase with increasing local nest density (Figure 5.4b); though this is not significant (G test for homogeneity: $G_{adj} = 1.63$, d.f. = 2, $p > 0.5$). In addition the mean proportion of cells per nest parasitised also increases with local nest density; however this is also not significant (G test for homogeneity: $G_{adj} = 1.73$, d.f. = 2, $p > 0.5$). Brood mortality thus appears to be density dependent when considering the proportion of nests and cells per nest parasitised. This can be further demonstrated by looking at the absolute numbers of parasitic larvae in nests and cells. The total number of parasitic larvae found in host nests is also related to the local nest density (Figure 5.5a: $y = 0.156x - 1.495$, $r^2 = 0.276$, d.f. = 18, $p = 0.017$). This shows that the number of successful ovipositions in a nest by Diptera increases with nest density, presumably due to the higher number of individuals present searching for host nests. At the highest densities this ensures that the majority of nests are parasitised, so that the total number of non-host larvae present is high.

The time window during which a cell can be parasitised is limited. For a successful oviposition, the cell needs to be suitably provisioned, but not yet sealed, and the female *H. rubicundus* needs to be absent from the nest. So if a cell is potentially available to parasitism it is likely that at high host/parasite densities, there would be multiple ovipositions by different female Diptera. We would therefore expect the number of larvae per cell to be higher at these densities; and this is the case (Figure 5.5b: $y = 0.026x - 0.115$, $r^2 = 0.230$, d.f. = 18, $p = 0.032$).

From the above it can be seen that occupying a nest in a densely aggregated area is associated with increased levels of parasitism by Diptera: an increased proportion of nests and cells attacked and larger numbers of larvae per nest and per cell. All are suggestive of directly density dependent parasitism. It would be interesting to collect a larger data set to resolve the significance of the relationship

in Figure 5.4. Analysis of a wider range of densities might also reveal any maximum in the rate of parasite activity at higher densities in accordance with Holling's functional responses (Holling 1959).

Parasitic larvae account for a high proportion of brood mortality in the *H. rubicundus* population at Invergowrie, so there is an obvious reproductive disadvantage in choosing to site a nest within a high density area. Therefore there must be some other factor(s) which account(s) for the persistence of this tendency to aggregate.

5.2.6 Philopatry

The tendency of individuals to return and found a new nest in close proximity to nest from which they emerged is well known in several species of Hymenoptera (section 1.2). Thus philopatry can account for both the formation and the maintenance of areas of high nesting density. Of the freshly emerged females marked between 1991 and 1993, seventeen were positively identified founding new nests the following season. The median dispersal distance was 1.37 m (range 0.07 to 7.09 m); however the distribution is strongly skewed to the left (Figure 5.6a).

To test whether the observed dispersal distances differed from those predicted by a random model it was necessary to generate a distribution of expected dispersal distances. This was done by using a computer to randomly pick two co-ordinates within the study site (42 x 3 m) and calculate the linear distance between them. This process was repeated a thousand times in order to generate a distribution of random dispersal distances (Figure 5.6b). Using a goodness of fit test (Fowler & Cohen 1992), it was found that the two distributions differed significantly ($\chi^2 = 90.99$, d.f. = 2, $p < 0.001$). It can therefore be concluded that the females are

returning closer to their the nest-site than would be expected if the site was of uniform quality and the females were nesting at random within it. This simple model does not however take into account variation in the quality of the nest-site which, as previously discussed, is an important factor in determining nest positioning. Females may be choosing to nest in areas of the most suitable substrate which happens to include their natal nests.

To disentangle philopatry and a preference for nesting in patches of favoured substrate would require further investigation. More detailed knowledge of the microscale variation in substrate parameters would be necessary as would a clear definition of good quality patch. Various alternate models of expected dispersal distances could then be proposed and tested against the observed patterns of dispersal. Such models might include: returning as close to the natal nest as possible, returning to a good quality point nearest to the natal nest, returning to the good quality patch containing the natal nest and returning to any good quality patch on the site. A tentative piece of evidence that females tend to return to within a patch is that 82 % of returning females had both natal and newly founded nests in the high density (high quality?) patch of quadrats 38B to 41B (Figure 2.4). This may, however, be a result of the sampling bias as this was the area most intensively studied and consequently females leaving this patch to nest elsewhere will have had a lower probability of being recorded.

One shortfall of any of these models is that they fail to take into account any females dispersing long distances to other sites. Consequently the analyses will only include those females showing nest-site fidelity and will ignore the tail of the distribution which includes very large dispersal distances.

Yanega (1990), concluded that philopatry was occurring in a US population of *H. rubicundus*; the mean dispersal distance was 0.32 ± 0.02 m (113) in a site much

smaller than the banking at Invergowrie. This study completely ignored any variation in nest site quality, and this conclusion may then be erroneous (for the reasons discussed above).

5.3 Nest-site selection across sites (UK)

A broad array of possible determinants for nest density across sites is given in Table 5.5. The number of factors that a multiple regression analysis is able to utilise is determined by the sample sizes used. As data from ten sites is available the maximum number of predictors that can be added into the equation is eight. Therefore three separate analyses were undertaken using the following categories: macro factors (hardness, aspect, slope, latitude, altitude, stone coverage, soil temperature excess and soil humidity), micro factors (as for the within site analysis) and weather factors (maximum and minimum temperatures, insolation and rainfall). If more sites were available it would be possible to combine these multiple regressions which would be statistically preferable, as the repeated use of multiple regressions are also subject to type I errors.

5.3.1 Soil hardness

For the macro factors the overall regression was non significant ($r^2 = 0.978$, $p = 0.320$) as were the individual factors (all $p > 0.151$). Although hardness itself was not a significant predictor of density in this analysis it was very important for the within site study and so further investigation is warranted. Figure 5.7a suggests that there may be a positive relationship between these two variables and this is substantiated by the regression in Table 5.5 ($y = 2.618x - 1.109$, $r^2 = 0.561$, d.f. = 8, $p = 0.013$). At relatively low nesting densities, there is a marked preference for softer soils within a site (Figure 5.1a). However, at higher densities the importance of maintaining the structural integrity of a nest becomes an important

factor, and the relationship between nest density and soil hardness changes when comparing the maximum nest densities from different sites (Figure 5.7a). As expected, when a female is electing to nest in an area with a number of conspecific nests already present, she chooses a harder substrate to initiate nest excavations.

The slopes of the regressions for within site and across sites are in different directions (Figure 5.8a). This however may be due to the types of data used: for the within site plot a range of all the occurring densities was used (0 to 37 nests m^{-2}); but for the across sites plot only the maximum recorded densities were used (up to 304 nests m^{-2}). It is therefore likely (especially for sites with low maximum densities) that within each site the relationship between hardness and density has a negative slope. A plot across sites would probably then consist of a series of negative slopes, with a positive relationship between maximum densities for each site and hardness.

Neighbouring nests are much more prone to collapsing into one another in softer soils. This becomes a very real problem when high densities lead to low mean NND; for instance 25.6 ± 1.1 mm (76) at the Kildale site. Maximum nest densities and NND are negatively related ($y = -33.014x + 110.443$, $r^2 = 0.559$, d.f. = 8, $p = 0.013$), see section 5.4.3. Furthermore, the relative spatial distribution of nests changes with increasing density such that they tend to have been sited in a less clumped manner. Choosing to found a nest at a point which maximises inter-neighbour distances could be another mechanism aiding nest structural integrity in addition to that of selecting a hard soil. The importance of the spatial distribution of nests will be discussed later in this chapter.

The hardness of a soil is largely accounted for by the proportion of gravel present (Fairbridge & Finkl 1979). It is therefore not surprising that the hardest soils contain the most gravel (Figure 5.8b: $y = 0.066x + 2.146$, $r^2 = 0.652$, d.f. = 8, $p =$

0.005). The micro factor multiple regression was non significant overall ($r^2 = 0.836$, $p = 0.099$) and all the predictors were highly non significant ($p > 0.414$) except for % gravel which was just non significant ($p = 0.086$); this suggests that the amount of gravel present may also be a good predictor of nest density (Table 5.5: $1.34 + 0.0205x$, $r^2 = 0.772$, $d.f = 8$, $p < 0.001$).

5.3.2 Aspect and slope

There are definite thermal advantages to nesting in a south facing slope and there is a strong preference for females to site nests in bankings with a southern aspect (Figure 5.7b). Taking magnetic north as 0° gives a mean angle of $188.2 \pm 32.0^\circ$ which is almost due south; this ensures maximal exposure to the sun throughout the day. To test whether the aspects are non-uniformly distributed, and whether they are significantly clustered around the predicted angle (180°) a modified Rayleigh test, or V test, was employed (Zar 1984). This showed that there was significant clustering around due south ($\mu_0 = 180^\circ$, $u = 3.224$, $n = 10$, $p < 0.0005$).

There is no relationship between slope and nesting density when looking across sites (multiple regression: $p > 0.151$). There may be preferences within some of these sites, as there was for the banking at Invergowrie, but this was not tested for. There was a large range of slope angles, 12.3 to 88.6° , with a mean of $55.9 \pm 9.3^\circ$ (Table 5.6). This indicates that *H. rubicundus* is tolerant of a wide range of slope angles, although most sites were far from flat and this may confer the thermal and drainage advantages outlined earlier.

5.3.3 Other abiotic factors

Table 5.5 shows a wide range of other abiotic factors none of which show significant correlations with maximum nest density (multiple regression results).

One might expect climatic variables, such as temperature, insolation and rainfall, to have an influence on nest-site suitability; this is not the case though (multiple regression: $r^2 = 28.3$, $p = 0.744$, all individual p values > 0.489). Undoubtedly they do influence nest densities, but the data used here are from local weather stations and reflect variation on a broad or national scale; on top of which there is a great deal of variation due to local conditions. It is therefore the microclimatic variation, as opposed to macroclimatic variation, that is responsible for the different suitabilities of particular sites. For instance, the lowest and highest mean maximum May air temperatures are 12.8 and 17.7 °C (Chatton and Swindon respectively); giving a total range of less than 5 °C (Table 5.6). On a microscale level, variation in aspect, slope and shading within a site can produce a local variation in air temperature of much more than 5 °C. *H. rubicundus* can readily nest within the range of climatic conditions found through the spread of field sites investigated, and so variations in these conditions have no influence on nest density. Altitude and latitude largely determine climatic conditions and so are subject to the same argument.

The properties of the substrate indicate the range, or at least part of it, that is suitable for nesting. Hardness and percentage gravel have already been discussed (section 5.3.1), and it is not surprising to find that the percentage of sand is significantly correlated with nest density since sand and gravel, being the two main components of soil, are inversely related. The soil categories used for nesting across sites are the same as those used within the Invergowrie site i.e. sand, loamy sand and sandy loam (Figures 5.9a and 5.9b). The only real differences are the Tentsmuir sites with more than 90% sand present, and the Prinited sites with higher combined silt and clay fractions. All these soil types help ensure that there is always a high water content without a risk of water-logging.

Soil temperatures, measured as the excess of ground temperature above that of air, are unrelated to nest density when comparing sites, but are important when looking within a site (Figure 5.2a). There is probably a tendency to pick the warmest area within a site, but the comparison across sites is confounded by the multitude of different factors that affect soil temperatures at each particular site.

5.3.4 *Sphecodes* and Diptera

The presence of kleptoparasitic *Sphecodes* sp. and/or parasitic Diptera result in some degree of brood mortality for *H. rubicundus*. Table 5.7 gives the maximum nest densities found at various sites within the UK, and also whether *Sphecodes* and Diptera are present or absent at that site. One would expect some sort of response in the nesting behaviour of the bees due to the differing levels of parasitism. Since it was not possible to excavate nests at most of the sites it is difficult to look directly at brood mortality. However there are significant differences in the maximum nest densities when comparing sites where the parasite is present and where it is absent.

For the Diptera the mean density is greater when the parasite is absent than when it is present (Figure 5.10a: two sample t test; $T = -2.68$, d.f. = 5, $p = 0.022$). It is important to note that with such a small sample size the significance of this result may be misleading. For instance when comparing the means of the three northern sites with the seven southern sites (Table 5.7), several other variables, in addition to nest density, also show significant differences (for instance mean temperature, size and soil hardness). These other factors are better examined using regression analysis which is a more powerful test than using a two sample t test to compare the group means. If data on the levels of parasitism for each site were available, then a different statistical test could be employed and would allow a more meaningful conclusion to be drawn.

If further study showed that sites with parasitic Diptera present did indeed have lower maximum nesting densities, then an explanation along the following lines could be invoked. A females fitness depends upon the level of brood mortality, which in turn is proportional to the local abundance of parasitic Diptera and local nest density (see section 5.2.5). It therefore follows that it is advantageous to nest in an area of slightly lower nest density than would be selected for if only purely abiotic factors were operating. So a female would be compromising her selection of the most suitable substrate and microclimatic factors in order to offset the cost of increased parasitism.

In contrast there is also a significantly higher nest density in areas where the cuckoo bee *Sphecodes* is present compared to those where it is not (Figure 5.10b: two sample t test; $T = -2.55$, d.f. = 7, $p = 0.019$). Again, due to the small sample size and type of data used, a cautious interpretation is required for the same reasons outlined above.

The difference in density could be accounted for by a dilution effect; whereby it is advantageous to nest in areas of high local density in order to minimise the probability of kleptoparasitic attack. Female *Sphecodes* search for host nests by investigating any burrow-like structures in a banking (whether they are *H. rubicundus* nests or not). When a suitable nest is located the kleptoparasite enters and destroys the hosts developing offspring and replaces them with her own eggs. The whole process takes considerable time, and a female *Sphecodes* is limited by the number of eggs present in her ovarioles (Iwata & Sakagami 1966). Therefore once a nest has been parasitised, its neighbours become relatively safe from attack by that cuckoo bee.

Sphecodes are nearly always found searching in areas of high nest density; thus there is a slightly elevated chance of a particular nest being attacked due to the increased number of searchers. However the relative abundance of the kleptoparasite is always very low when compared to that of the host nests (pers. obs.) so that the chance of any individual nest being attacked is very small. The combined result of these two probabilities is an overall advantage in nesting in an area of high local nest density i.e. there is a beneficial dilution effect because parasitism is inversely density dependent.

5.3.5 Other biotic factors

Maximum nest density and mean nest density over the whole site are closely related (Figure 5.11a: $y = 0.020x + 1.331$, $r^2 = 0.479$, d.f. = 8, $p = 0.027$). This probably indicates that the pressure to nest in very dense parts of an aggregation is a function of the overall nesting density of the site. Nest density thus depends on the suitability of a particular area, so that the maximum and the mean densities will both depend upon the total number of nesting females attempting to use a limited area. It follows that the relationships between mean nest density and the various biotic and abiotic factors examined are very similar to the relationships found for maximum nest density.

Neither total nest area nor the total number of nests present within an aggregation are related to the maximum nest density (Table 5.5). However, the total number of nests and the total area of an aggregation are highly correlated (Figure 5.11b: $y = 0.697x + 1.563$, $r^2 = 0.806$, d.f. = 8, $p < 0.001$). This suggests that at a particular site there is some sort of basic carrying capacity of the ground; such that, irrespective of the finer grades of suitability of a site, the total number of nests will ultimately depend on the total area available.

Female size (head width) might be a factor influencing the density of nests in different aggregations. The range of head widths is considerable (Table 5.6); from 2.00 to 2.91 mm. Indeed, larger females will construct larger nests which might require bigger NND to maintain structural integrity, which in turn would produce lower densities. However, from the available data, nest density is not correlated with female size (Table 5.5: $y = 0.62 + 0.471x$, $r^2 = 0.095$, d.f. = 8, $p = 0.421$).

5.4 Spatial distribution of nests

5.4.1 Measurement of spatial distributions and some common factors influencing nest spacing

The relative spacing of objects within two areas of equal density can be markedly different. Clark & Evans (1954, 1956) developed the 'distance to nearest neighbour' as a measure of spatial relationships within a population. Essentially what this method does is to compare the mean of the measured NNDs with the mean that would be expected if the points were distributed purely at random. The expected mean NND is simply a function of the density of objects within a given area, which is given by the formula:

$$\frac{1}{2} \sqrt{\text{density}}.$$

The ratio of the observed and expected NNDs is then a measure of the relative spacing of the points; I have used the term 'dispersion index' for this ratio. If the spacing was purely random then the observed and expected NNDs would be the same and so the dispersion index would be exactly one. A value less than one indicates clumping, and a value greater than one indicates a regular pattern of spacing. The dispersion index can then be tested to see if it is significantly different from one, thus giving the test statistical validity.

The problem of edge effects, which produce a bias towards regularity, was avoided by excluding those individuals in the sample that were closer to the boundary than they were to their nearest neighbours. Use of every individual in a sample, as opposed to a randomly selected sub-sample, means that the NNDs are not independent since paired individuals are each others nearest neighbours. The effect on the Clark and Evans test is negligible but bias is introduced into other tests, such as the Thompson test, which precludes its use for this type of spatial analysis (Campbell 1990).

Quadrat size can seriously affect the detection of the correct type of dispersion pattern (Poole 1974). Too large a quadrat will result in a patchily clumped aggregation being interpreted as being more dispersed than it really is (Greig-Smith 1952). Similarly, regular patterning within a clump will be missed unless a quadrat of similar size to a local cluster is used. To overcome this two sizes of quadrats were used in the NND analysis (1 m^2 and 0.01 m^2).

The NND method has been applied to a wide range of organisms (reviewed by Southwood 1978). For hymenopteran nests it has been used by Rubink (1978) who showed the nests of *Bembix pruinosa* have a dispersion index significantly greater than one. He attributed this regular spacing to the selection of new nest-sites by females, such that aggressive interactions between females in neighbouring nests was minimised.

Another commonly used test is Morisita's index (Poole 1974) which also gives a measure of dispersion. However one of the assumptions for this test (that individuals within a clump are randomly spaced) is not satisfied and therefore the test could not be used.

There are a wide range of factors that could account for the different densities within an aggregation, and that could also account for different patterns of nests spacing (Table 5.1). If only one factor is operating then it is possible to make simple predictions about nest spacing, and these are outlined below.

If females are exhibiting philopatric behaviour then one might expect an aggregation to build up over time as subsequent generations of bees attempt to nest in close proximity to their natal nests. The spacing of nests would be expected to be random (Yanega 1990), except in the extreme case where offspring tried to nest exceptionally close, i.e. almost touching the maternal nest, when there would be a very localised clustering.

Limited substrate would also produce areas of high density, as bees selected the most suitable areas to nest in. Within a favoured area spacing would be random; however when compared with a less favourable area it would appear more clumped. Whether an area contains a clump or not then depends upon the size of the sample unit.

Mortality due to parasitism and/or predation would have an effect on nest locations. If it were density dependent, then it would be advantageous to nest away from other nests in order to reduce the risk, and so a low density aggregation (or solitary nesting) would be expected. Inversely density dependent parasitism would have the opposite effect and so would favour high density aggregations. If mortality were density independent then one would not expect any particular density to be favoured. Rosenheim (1990) summarises these three types of density related parasitism for 13 studies of aggregations of ground nesting Hymenoptera.

Selfish herding is one possible response to parasite or predator pressure, whereby central nests have a reduced risk of attack relative to peripheral nests (section 1.2); however this relies upon differential mortality depending upon the position of a nest within an aggregation, and not the local density within which it is found. A clumping of nests would therefore be expected as the centripetal tendency favoured the siting of nests closer together. This phenomenon has, however, never been conclusively shown to occur in hypogeous Hymenoptera, although it has been suggested by Wcislo (1984) and Larsson (1986).

Group defence is another response to parasitism whereby several nests in close proximity allow the occupiers to defend their nests more effectively by aiding each other. This may occur directly in some sort of aggressive encounter (Batra 1978) or indirectly where individuals from a neighbouring nest disturbs a parasites activity by simply entering or leaving their own nest. This kind of defence would be facilitated by high nest densities and clumped spacing of nests.

The regular spacing of nests could result from one of several factors that favour the maximisation of inter neighbour distances. Maintaining nest structure is one (discussed in sections 5.2.1 and 5.3.1). Conspecific aggression has also been invoked as an explanation (Rubink 1978; Brockmann 1979). Fungal infection and nematode attack can also be significant causes of brood mortality in hypogeous hymenoptera (Roubik 1989); it may be that nests close to already contaminated neighbours may be more prone to infection. Although this has yet to be properly shown, it could be another factor accounting for a regular spatial distribution of nests.

It should be noted that more than one factor may be operating at once, and so predictions based purely on density and dispersion index measurements are

unreliable. However detailed knowledge about the biology of an organism being studied can allow a much more accurate explanation of nesting patterns.

5.4.2 Within site

The mean NNDs remain fairly constant for the range of nest densities observed at Invergowrie (Figure 5.12a: $y = -11.009x + 87.205$, $r^2 = 0.062$, d.f. = 14, $p = 0.335$). At the highest densities there is little difference between the observed and the expected NNDs, which is indicative of random nest spacing. However at lower densities the difference between the observed and the expected NNDs is much more marked.

Dispersion index and nest density are highly correlated (Figure 5.12b: $y = 0.396x + 0.138$, $r^2 = 0.853$, d.f. = 14, $p < 0.001$), this is however an artefact resulting from the way the dispersion indices were calculated rather than any real differences in the spatial distribution of nests. As observed NNDs remain approximately constant across a range of densities (see above), and expected NNDs increase with decreasing density this results in a decrease in the dispersion index at low densities. In other words the nests are relatively more clumped at lower densities when the total available space is considered, but the actual spatial distribution will be no different to those at higher densities. Within each quadrat there will be patches of locally favoured substrate that are being preferentially selected by nest founders, and these will tend to become filled first before other less favourable sub-patches are used. The overall pattern is then one of nests aggregated into patches, with areas of higher density having larger patches or more of them.

There are three possibilities that can account for this clumped spatial distribution. Firstly, selfish herding; but this can be discounted since Diptera follow a host to a nest and do not enter the first nest they find when approaching an aggregation.

Secondly, passive group defence (whereby a parasite is incidentally disturbed by a neighbouring hosts activity); but this is so infrequent that it cannot account for the favouring nest clustering (pers. obs.). Therefore the clumping must be due to the heterogeneous nature of the substrate. There are likely to be very fine grained variations in substrate properties, such as hardness and ground temperature, within the 1m² quadrats; and these would be undetected in the preceding analysis. These small scale changes would then influence the suitability of areas within each quadrat, with females selecting the most favourable sub-patches within a particular patch. Thus, depending upon the micro-variation in these substrate properties, the distribution of nests will be either clumped or random as predicted.

In order to look at the spacing between nests on a smaller scale, three 0.01 m² quadrats were selected within visually obvious clumps within the 1 m² quadrat of highest density; and then the same NND analysis carried out. The mean observed NND was 36.8 ± 3.7 mm and the dispersion index was 2.55 which was highly significant ($p < 0.001$). Therefore on this scale the nests were regularly spaced, suggesting that within the clumps of nests there is a tendency to maximise inter-neighbour distances.

Of the three factors that could explain this (Table 5.1), only one is supported by good evidence. Conspecific aggression was never observed between females of *H. rubicundus*; and there was very little incidence of brood mortality due to fungal or nematodal attack. Batra (1965) found that 1.9 % of *L. zephyrum* cells contained mats of mycelium; and Packer *et al.* (1989a) reported that 1.0 % of *Lasioglossum comagenense* cells had some sort of microbial infection. Less than 5 % of excavated cells at Invergowrie contained pollen balls with any fungus present (pers. obs) and this is still relatively small when compared to mortality due to Diptera parasitism. But the importance of maintaining structural integrity is borne out by the

arguments in sections 5.2.1 and 5.3.1. Further evidence comes from the relationship between hardness and observed NNDs (Figure 5.14a: $y = -0.013x = 2.556$, $r^2 = 0.117$, d.f. = 14, $p = 0.182$). One would expect a negative relationship to exist here, such that the softest soils force nests to be less clumped; although the regression is non significant the trend is in the right direction. The areas with the lowest NNDs (< 60 mm) all have hardness values above 1.5 Kgf cm⁻², which may reflect the minimum soil strength necessary to support cells that are ≈ 30 mm away from the burrow at these low NNDs found within the Invergowie site (section 5.2.1).

A mechanism by which females choose to nest at a particular point can be proposed. A patch of the most suitable substrate will be favoured, and a searching female may visually inspect an area for the presence and position of other nest entrances. Further, information about the hardness of the soil may be obtained by biting the surface and carrying out a test dig (section 5.1.2). These two parameters will be contributors in determining the overall suitability of a particular point, and a female may use these in deciding whether to initiate a nest or not. If a foundress elects to start a nest then information about the location of adjacent nests may be important in regulating lateral length. This would require experimental testing in order to be verified, by manipulating local nest density and/or hardness and then excavating nests to measure lateral lengths.

5.4.3 Across sites

NNDs are negatively correlated with maximum nest densities (Figure 5.13a: $y = -33.014x + 110.443$, $r^2 = 0.559$, d.f. = 8, $p = 0.013$) such that higher densities have lower NNDs, as we might expect. For the highest densities the observed and expected NNDs are very similar, but for lower densities the observed are all lower than the expected. This difference means that the low maximum density sites

tend to have more clumped spatial distributions than the high density sites, which possess more random arrangements. There may be a positive trend between density and dispersion index across sites but with these data it is non significant (Figure 5.13b: $y = 0.310x + 0.228$, $r^2 = 0.362$, d.f. = 8, $p = 0.066$).

Table 5.8 shows that six out of ten of the sites have dispersion indexes significantly different from one. There would therefore appear to be some sort of advantage to nesting in a clumped manner. The same arguments used to explain clumping within a site can be invoked here; selfish herding and group defence can be rejected for the same reasons as before, so that again selection of the most favourable substrate on a microscale level by the females has presumably led to an overall tendency to aggregate.

Parasitism may be density dependent (Section 5.2.5), but there is no difference in the mean NNDs and dispersion ratios between sites where the parasites are present or absent (Table 5.9). Parasitic pressure can then not be used to explain differences in the spatial distributions of nests.

Consequently another factor must be responsible for the random nature of nest spacing observed in the other four sites. As for the previous section, of the factors accounting for more regular spacing of nests, only the need to keep a nests structural integrity intact would explain this satisfactorily. Kildale has a very high density (304 nest m^{-2}) and so regular nest spacing is necessary (section 5.3.1). The two Prinsted sites have similar densities to Newcastle and Chatton but are not clumped; they do however possess relatively soft soils when compared to the latter two sites, and so the nests will be selected at less clustered areas. Invergowrie has some of the softest soils overall and so greater spacing might be expected anyway.

Overall then, the four sites with random spacing probably result from selection favouring more regular spacing which has reduced the advantages of clustering within patches of the most suitable substrate, thus leading to an intermediate state of spatial patterning.

Though there is no overall significant relationship between NNDs and hardness (5.14b: $y = -0.039x + 5.497$, $r^2 = 0.239$, d.f. = 8, $p = 0.186$), when all ten sites are considered, there appears to be a negative relationship when NNDs below 50 mm are examined. The site with the softest soil within this range (Te) has the highest NNDs, while sites with harder soils have lower NNDs. Above NNDs of 50 mm hardness may not be important in directly determining NNDs through its influence on nest structural stability. However, at NNDs less than 50 mm, nest spacing becomes so tight that only substrate of sufficient hardness can support nest structures properly. In patches of the highest quality soil there will be a tendency of nests to be packed together as much as is possible. At densities where NNDs are greater than 50 mm there are unlikely to be problems of maintaining nest architecture, but below this threshold closer nest spacing (smaller NNDs) can only occur where the substrate is hard enough. This general relationship is in accordance with the findings for within the Invergowrie site.

5.5 Summary

H. rubicundus is able to utilise a range of edaphic and microclimatic conditions when choosing a site to excavate a nest (Table 5.6). There are some factors with broad tolerances such as slope and hardness, and others with much narrower limits such as aspect, soil humidity and particle composition.

However within these observed ranges there are definite preferences for certain conditions which lead to patches of high nest density. In other words there are limited areas of substrate with the most desirable characteristics for nesting in.

There is a preference for softer soils, that are easier to dig, within a site with a low overall density; but in much denser aggregations problems of maintaining the structural integrity of a nest lead to the favouring of harder soils. The thermal advantages of having a warm nest mean that the most suitable areas are those with a southern aspect and a slope which receives the maximum amount of solar radiation.

Prolonged searching for a suitable nest-site may be an expensive activity in terms of time and energy, so that any behaviour that can make this process more efficient will be selected for. Females from the previous season may learn the position and patch quality of their natal nest, and then return to that area when founding their own nests. This kind of limited dispersion behaviour is thus likely to evolve when there is a fitness advantage in selecting the most favourable nest-sites in a patch of variable quality.

Within the Invergowrie site, mortality due to parasitism is directly density dependent which would select for less aggregated nest assemblages. There are then two possibilities which could account for the fact that areas of high density still exist. First, the increase in fitness due to selecting the most favourable substrate is greater than the loss of fitness due to parasitic attack. This is clearly not the case since there is no difference in the number or size of offspring at different nest densities. The second possibility is that the aggregation is in fact declining on the whole. This process may take many years and is not apparent from my short term study. The aggregation may well have been established and grown to its current size relatively free of parasites, as it will take several seasons

for the parasites to find an aggregation and build up a population sufficient to cause this level of mortality. This founding, growth and death of a hymenopteran aggregation is well documented (Michener 1974) for several species of host and parasite.

Table 5.1. Summary of predicted nest densities and nest spacing for various factors influencing nest site selection for *H. rubicundus*. The predictions made are for cases where only a single factor is operating. The effective range only suggests the order of magnitude of the distances that a particular factor is likely to act through. [†] Indicates that a lower density may result if the influence is very strong.

<u>Factor</u>	<u>Density</u>	<u>Spacing</u>	<u>Range</u>
Philopatry	high	random	cm - m
Limited substrate	high	random/clumped	cm - m
Parasitism/predation:			
Density dependent	low	random	cm - m
Inversely density dependent	high	random	cm - m
Density independent	no influence	random	cm - m
Selfish herding	high	clumped	cm
Group defence	high	clumped	mm - cm
Structural integrity	no influence	regular	mm
Conspecific aggression	low [†]	regular	mm
Infection spread	low [†]	regular	mm

Table 5.2. Summary of results for various abiotic factors regressed against nest density (nests m⁻²) for the aggregation at Invergowrie.

<u>Factor</u>	<u>Equation</u>	<u>R²</u>	<u>d.f.</u>	<u>p value</u>
Hardness (Kgf/cm ²)	2.252 - 0.037x	0.339	40	0.000
Slope (°)	20.198 + 0.118x	0.114	40	0.024
Stone coverage	1.386 - 0.002x	0.028	19	0.420
Surface temperature (°C)	27.184 - 0.028x	0.105	40	0.021
Soil temperature (°C)	30.641 + 0.004x	0.005	40	0.605
Soil humidity (%)	80.501 - 0.001x	0.000	40	0.896
Gravel (%)	34.1 - 0.262x	0.043	11	0.496
Sand (%)	21.6 - 1.14x	0.006	11	0.809
Silt and Clay (%)	7.1 + 0.289x	0.049	11	0.468
Organics (%)	36.5 - 319x	0.011	11	0.764
pH (-log ₁₀ [H ⁺])	-68.9 + 12.1x	0.047	40	0.169
Inorganic salts (μS)	14.5 + 0.82x	0.003	40	0.730

Table 5.3. Multiple regression analysis of density within the Invergowrie nest site: (a) Macro factors. T_g = surface temperature, T_{soil} = soil temperature, RH_{soil} = soil RH, In = inorganic salts (b) Micro factors.

A. Macro factors

Regression equation: $Density = 15.3 + 1.14 \text{ Slope} - 9.43 \text{ Hardness} + 2.70 T_g - 2.07 T_{soil} + 0.088 RH_{soil} - 3.33 \text{ pH} - 1.71 \text{ In}$, $r^2 = 0.534$, $p < 0.001$

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	15.34	81.73	0.19	0.852
Slope	1.1447	0.3688	3.10	0.004
Hardness	-9.429	2.023	-4.66	0.000
T_g	2.704	1.531	1.77	0.086
T_{soil}	-2.067	2.300	-0.90	0.375
RH_{soil}	0.0883	0.3590	0.25	0.807
pH	-3.325	7.269	-0.46	0.650
In	-1.707	1.856	-0.92	0.364

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	7	3061.38	437.34	5.57	0.000
Error	34	2668.24	78.48		
Total	41	5729.62			

B. Micro factors

Regression equation: $Density = -296 - 1.07 \text{ Stones} + 4.04 \% \text{ Gravel} - 513 \% \text{ Organic} + 3.42 \% \text{ Sand}$, $r^2 = 0.191$, $p = 0.833$

Note: % Sand + Clay was removed from the equation as it is highly correlated with other predictor variables.

<u>Predictor</u>	<u>Coef.</u>	<u>S.D.</u>	<u>t-ratio</u>	<u>p</u>
constant	-296.0	620.1	-0.48	0.650
Stones	-1.066	1.297	-0.82	0.443
% Gravel	4.042	6.414	0.63	0.552
% Organics	-513	1168	-0.44	0.676
% Sand	3.423	6.184	0.55	0.600

<u>Source</u>	<u>d.f.</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>p</u>
Regression	4	534.0	133.5	0.35	0.833
Error	6	2262.2	377.0		
Total	10	2796.2			

Table 5.4. Means \pm SE of various factors measured in four areas of different nest density at Invergowrie. The units for the factors are all as before. In all cases $n = 5$. Measurements made on 2.8.94 at 15:00; $T_a = 23.5^\circ\text{C}$, $\text{RH} = 48.8\%$.

<u>Factor</u>	<u>O Density 1</u>	<u>O Density 2</u>	<u>Low Density</u>	<u>High Density</u>
Nest density	0	0	15.2 ± 4.0	28.8 ± 6.0
Hardness	4.55 ± 0.09	4.40 ± 0.13	2.05 ± 0.09	1.65 ± 0.13
Slope	3.4 ± 1.0	18.6 ± 0.7	26.8 ± 0.7	29.8 ± 0.9
Surface temp.	29.02 ± 0.09	29.10 ± 0.10	29.72 ± 0.07	30.28 ± 0.14
Soil temp.	27.90 ± 0.10	27.94 ± 0.10	27.80 ± 0.06	28.42 ± 0.15
Soil humidity	87.14 ± 0.46	85.58 ± 0.34	86.60 ± 0.54	86.64 ± 0.27
Vegetation cover	26.0 ± 1.87	0	0	0
Stone coverage	9.4 ± 1.2	28.2 ± 2.8	44.6 ± 2.2	25.2 ± 2.5

Table 5.5. Summary of results for various factors regressed against maximum nest density [\log_{10} (nests m^{-2})]; all with 8 d.f.. '+' indicates that the variable is normally distributed; 'log' indicates that the variable is normally distributed after a \log_{10} transformation.

<u>Factor</u>	<u>Normal</u>	<u>Equation</u>	<u>R²</u>	<u>p value</u>
Hardness (Kgf cm^{-2})	+	$1.00 + 0.214x$	0.561	0.013
Aspect (°)	+	$1.97 - 0.00620x$	0.171	0.234
Slope (°)	+	$1.84 - 0.00183x$	0.016	0.724
Latitude (')	+	$1.88 - 0.0058x$	0.037	0.596
Altitude (m)	log	$1.73 + 0.003x$	0.000	0.988
Vegetation cover (%)	+	$1.62 + 0.0141x$	0.143	0.280
Stone coverage	+	$1.76 - 0.0086x$	0.020	0.699
Soil temp. excess (°C)	+	$2.12 - 0.107x$	0.165	0.244
Soil humidity (%)	+	$4.21 - 0.0302x$	0.170	0.236
Gravel (%)	+	$1.34 + 0.0205x$	0.772	0.000
Sand (%)	+	$2.94 - 0.0160x$	0.568	0.010
Silt & Clay (%)	log	$1.62 + 0.160x$	0.034	0.634
Organics (%)	+	$1.96 - 4.38x$	0.108	0.387
pH ($-\log_{10}[\text{H}^+]$)	+	$3.24 - 2.04x$	0.031	0.629
Inorganic salts (μS)	+	$0.874 + 0.430x$	0.230	0.158
Colour hue	+	$2.71 - 0.103x$	0.149	0.271
Colour chroma	+	$1.91 - 0.045x$	0.014	0.744
Colour value	+	$1.39 + 0.107x$	0.214	0.178
Max temp. (°C)	+	$3.39 - 0.091x$	0.058	0.503
Min temp. (°C)	+	$1.35 + 0.0379x$	0.019	0.705
Insolation (h)	+	$1.46 + 0.0014x$	0.002	0.891
Rainfall (mm)	+	$3.02 - 0.0273x$	0.102	0.369
Female size (mm)	+	$0.62 + 0.471x$	0.095	0.421
Mean nest density	+	$1.33 + 0.0199x$	0.478	0.027
Total nest area (m^2)	log	$1.77 - 0.024x$	0.002	0.905
Total nest number	log	$1.23 + 0.212x$	0.086	0.412
NND (mm)	+	$2.63 - 0.0168x$	0.559	0.013
Dispersion Index	+	$0.845 + 1.17x$	0.365	0.066

Table 5.6. Mean, standard error, minimum and maximum values for various factors measured at 10 UK field sites in May 1993. [†] indicates that the mean and standard error have been calculated from the log₁₀ transformed data then the result back transformed.

<u>Factor</u>	<u>Mean</u>	<u>SE</u>	<u>Min.</u>	<u>Max.</u>
Maximum density (nests m ⁻²) [†]	54.7	1.4	18.0	304.0
Hardness (Kgf cm ⁻²)	3.4	0.5	1.7	5.5
Aspect (°)	189.4	14.8	120.0	261.0
Slope (°)	55.9	9.3	12.3	88.6
Latitude (')	243.4	43.8	50.0	387.0
Altitude (m) [†]	11.6	1.4	1.0	180.0
Vegetation cover (%)	8.5	3.6	0.0	30.0
Stone coverage	2.4	2.4	0	24
Soil temp. excess (°C)	3.54	0.51	1.40	5.80
Soil humidity (%)	81.7	1.8	73.0	88.5
Gravel (%)	19.7	5.7	0.1	60.7
Sand (%)	75.6	6.4	37.6	99.2
Silt & Clay (%) [†]	2.6	1.4	0.9	28.0
Organics (%)	0.063	0.010	0.030	0.120
pH (-log ₁₀ [H ⁺])	7.4	0.1	7.0	8.2
Inorganic salts (μS)	2.01	0.15	1.00	3.00
Colour hue	9.5	0.5	5.0	10.0
Colour chroma	4.0	0.4	2.0	5.0
Colour value	3.2	0.6	1.0	6.0
Max temp. (°C)	15.2	0.5	12.8	17.7
Min temp. (°C)	7.5	0.4	5.7	9.5
Insolation (h)	218.4	6.9	192.0	252.7
Rainfall (mm)	24.8	2.2	18.7	36.6
Female size (mm)	2.48	0.09	2.00	2.91
Mean nest density (nests m ⁻²)	20.4	4.6	4.8	44.8
Total nest area (m ²) [†]	14.8	1.7	2.0	240.0
Total nest number [†]	239.3	1.5	36	3280
NND (mm)	53.0	5.9	25.2	80.4
Dispersion Index	0.77	0.07	0.48	1.11

Table 5.7. Presence or absence of *Sphecodes* and Diptera and maximum nest densities [$\log_{10}(\text{nests/m}^2)$] at 10 field sites in the UK. '+' indicates parasite present; '-' indicates parasite absent; '?' indicates unknown whether parasite is present or absent.

<u>Site</u>	<u>Sphecodes</u>	<u>Diptera</u>	<u>Nest density</u>
Invergowrie	-	+	1.57
Tentsmuir east	-	+	1.40
Tentsmuir west	-	+	1.26
Chatton	+	-	1.70
Newcastle	+	?	2.20
Kildale	+	-	2.48
Gibraltar Point	+	-	1.60
Swindon	+	?	1.28
Prinsted south	+	-	1.68
Prinsted north	+	-	2.21

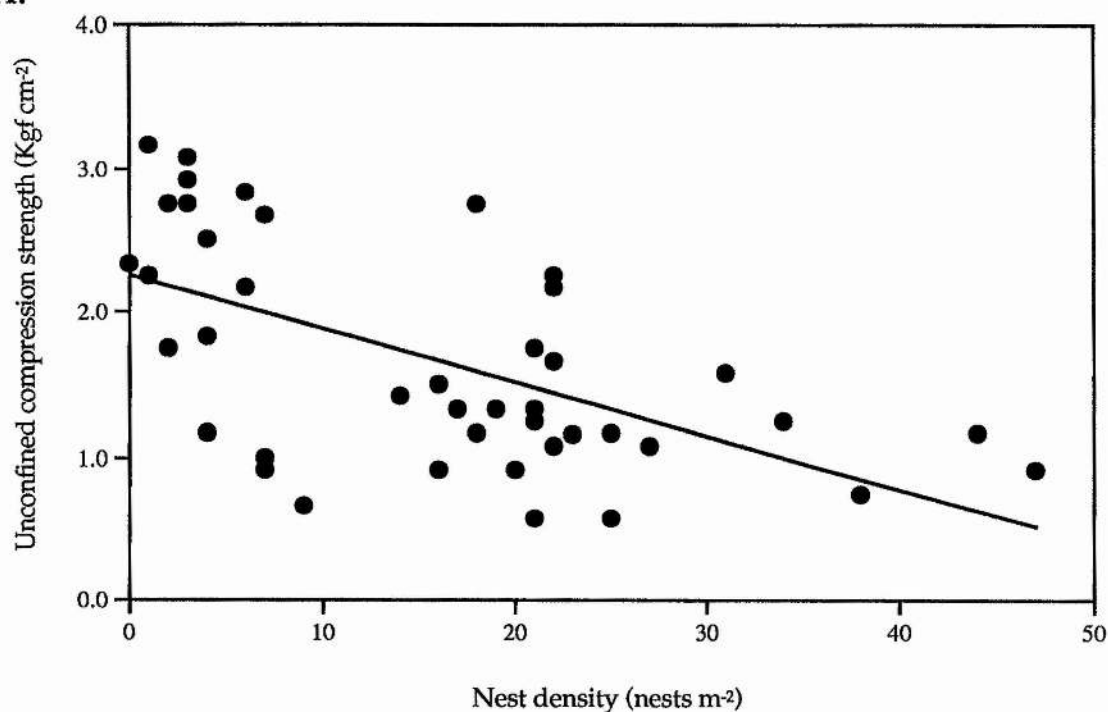
Table 5.8. The maximum nest density (nests m⁻²), mean observed nearest neighbour distances (mm) and dispersion index (with significance of deviation from 1.00) for 10 field sites in the UK. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns non-significant.

<u>Site</u>	<u>Max density</u>	<u>Obs. NND</u>	<u>Disp. Index</u>	<u>Sig.</u>
Invergowrie	37	68.3 ± 4.8	0.83	ns
Tentsmuir east	25	48.0 ± 4.9	0.48	***
Tentsmuir west	18	69.7 ± 10.4	0.59	**
Chatton	51	53.7 ± 5.5	0.76	**
Newcastle	160	32.3 ± 2.6	0.82	*
Kildale	304	25.2 ± 1.1	0.88	ns
Gibraltar Point	40	39.9 ± 7.3	0.50	**
Swindon	19	71.4 ± 7.7	0.63	**
Prinsted south	48	80.4 ± 6.2	1.11	ns
Prinsted north	164	41.3 ± 4.1	1.06	ns

Table 5.9. Summary of means for three measures of spatial distribution of nests; and results of two sample t tests for differences in site means with and without (a) parasitic Diptera and (b) *Sphecodes*.

(a) <u>Diptera</u>					
<u>Measure</u>	<u>Present</u>	<u>Absent</u>	<u>T value</u>	<u>d.f.</u>	<u>p value</u>
Maximum density	1.41 ± 0.09	1.93 ± 0.17	-2.68	5	0.022
Nearest neighbour distance	62.0 ± 7.0	48.1 ± 9.3	1.20	5	0.140
Dispersion Index	0.63 ± 0.10	0.86 ± 0.11	-1.51	5	0.095
(b) <u>Sphecodes</u>					
<u>Measure</u>	<u>Present</u>	<u>Absent</u>	<u>T value</u>	<u>d.f.</u>	<u>p value</u>
Maximum density	1.88 ± 0.16	1.41 ± 0.09	-2.55	7	0.019
Nearest neighbour distance	49.2 ± 7.7	62.0 ± 7.0	1.23	6	0.130
Dispersion Index	0.82 ± 0.08	0.63 ± 0.10	-1.43	4	0.110

A.



B.

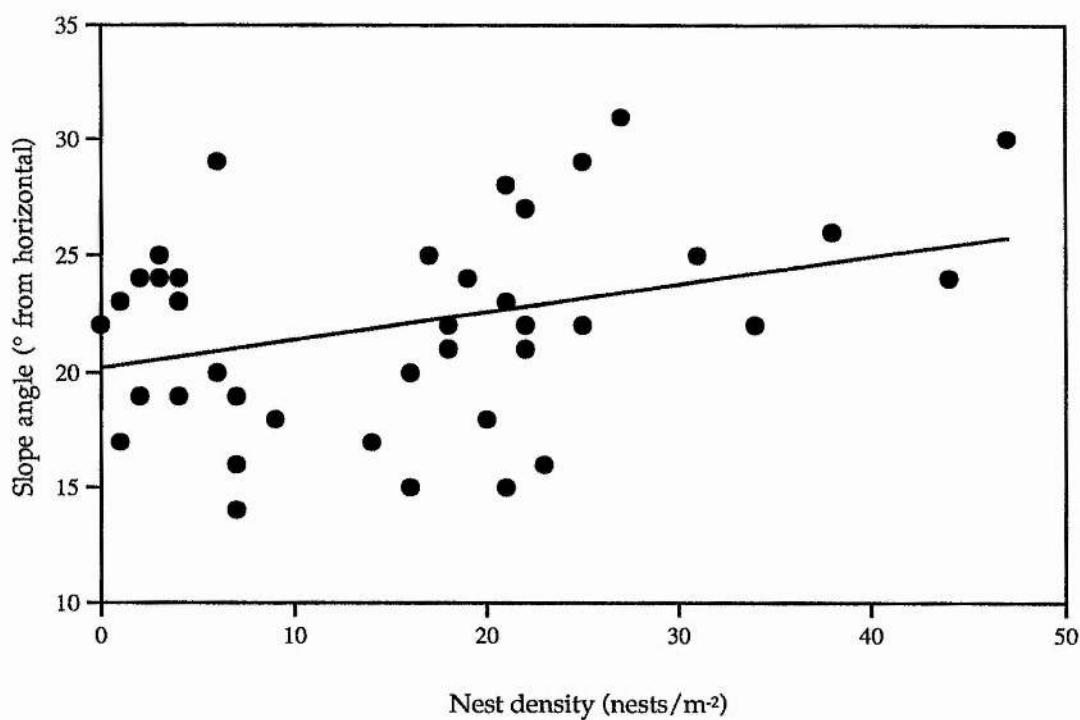
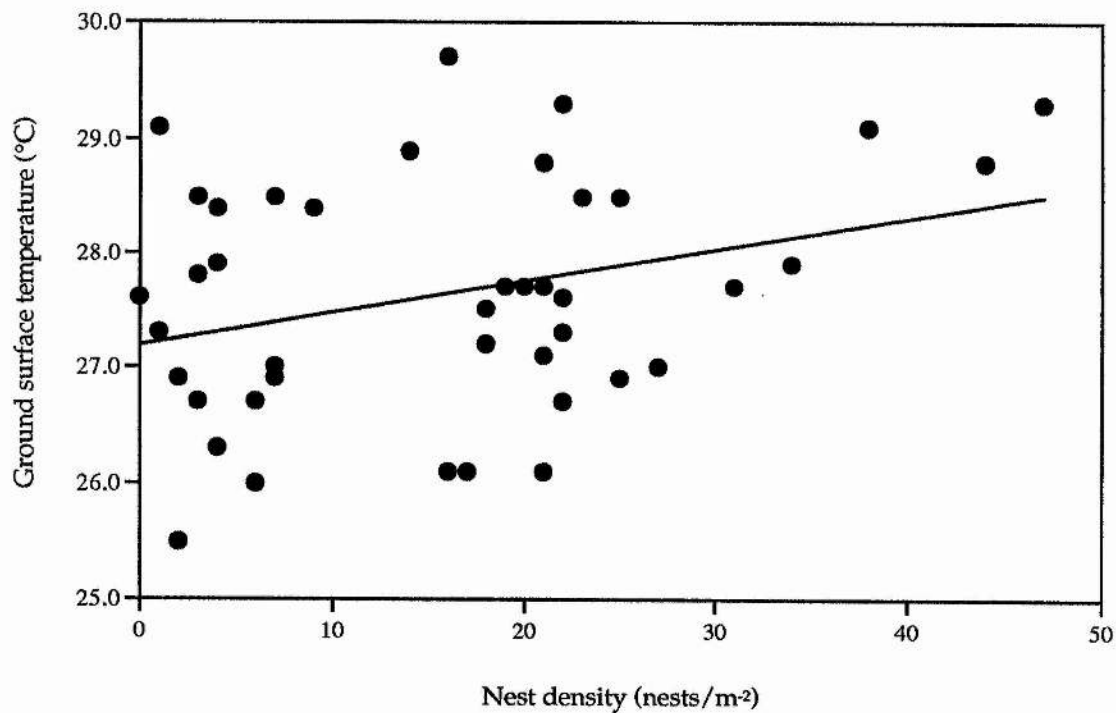


Fig 5.1. (a) Soil hardness and nest density ($y = -0.037x + 2.252$, $r^2 = 0.339$, d.f. = 40, $p < 0.001$). **(b)** Slope angle and nest density ($y = 0.118x + 20.198$, $r^2 = 0.114$, d.f. = 40, $p = 0.024$).

A.



B.

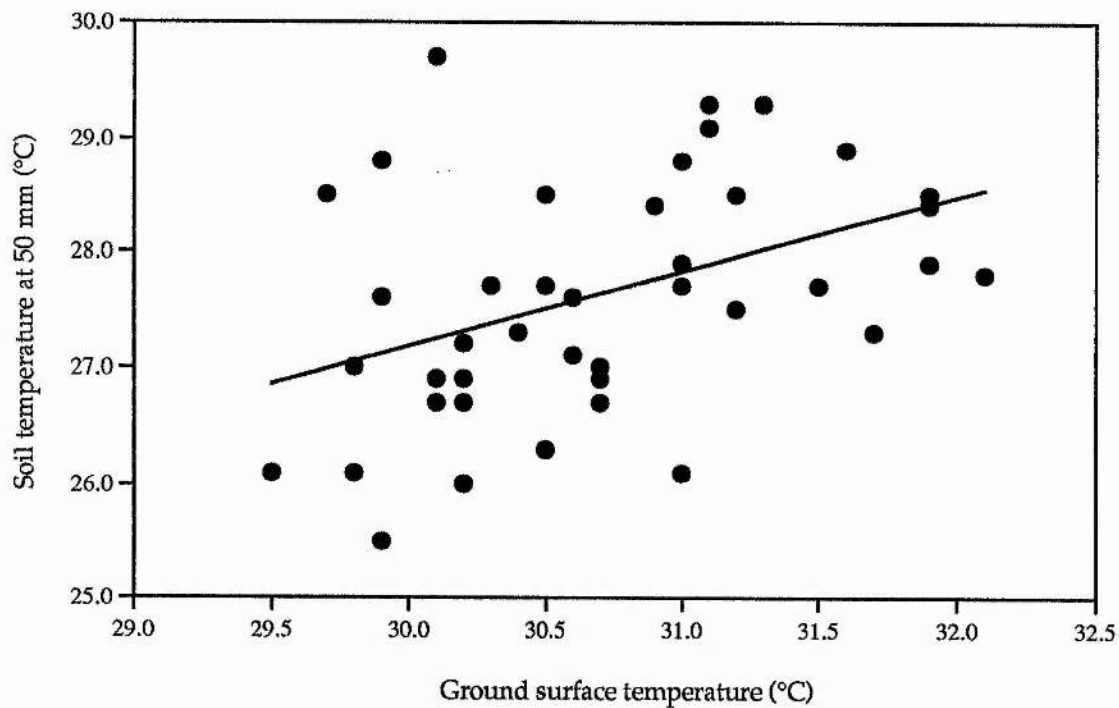


Fig 5.2. (a) Temperature of ground surface and nest density ($y = 0.028x + 27.184$, $r^2 = 0.105$, d.f. = 40, $p = 0.021$). (b) Soil temperature at 50 mm and soil surface temperature ($y = 0.649x + 7.701$, $r^2 = 0.179$, d.f. = 40, $p = 0.005$).

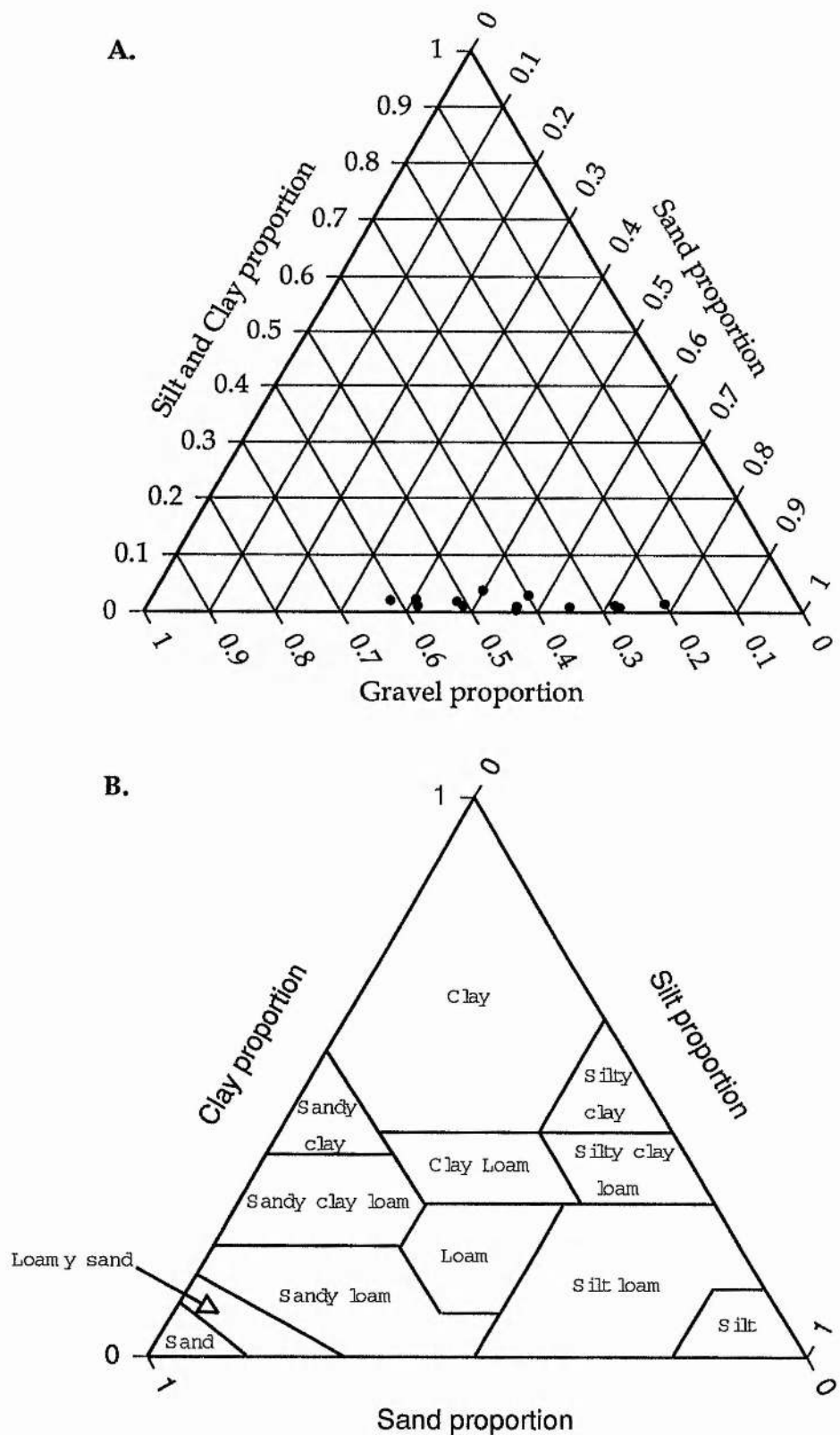


Fig 5.3. (a) Textural classification diagram showing the relative proportions of gravel, sand and silt & clay found in the surface soil of various quadrats within the aggregation at Invergowrie ($n = 13$). (b) Texture classes according to the British Standard System.

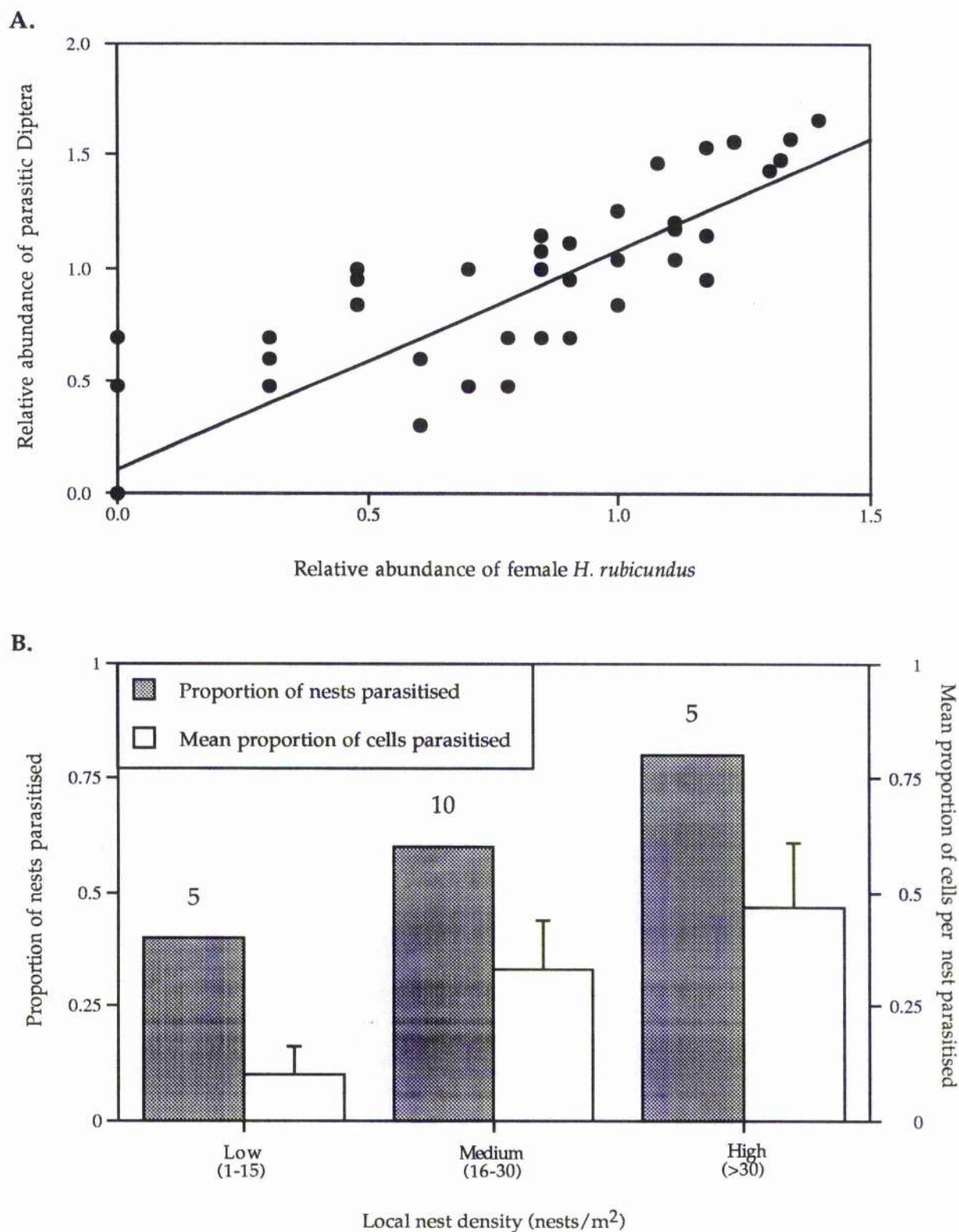
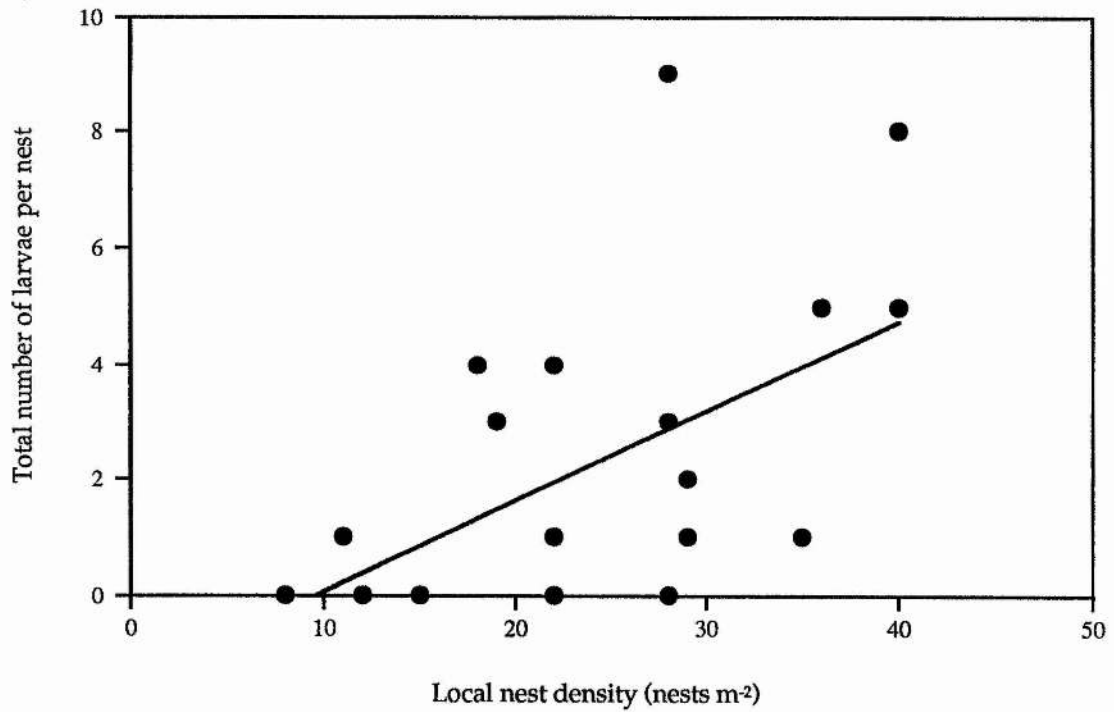


Fig 5.4. (a) Relative abundances of parasitic diptera and *H. rubicundus* females, measured as number of individuals 10 minute⁻¹ period m⁻² and transformed using Log₁₀(x+1); ($y = 0.975x + 0.106$, $r^2 = 0.762$, d.f. = 70, $p < 0.001$). **(b)** Proportion of nests containing at least one dipterous larva and mean number of cells per nest parasitised at three different densities; numbers above bars indicate the number of nests excavated.

A.



B.

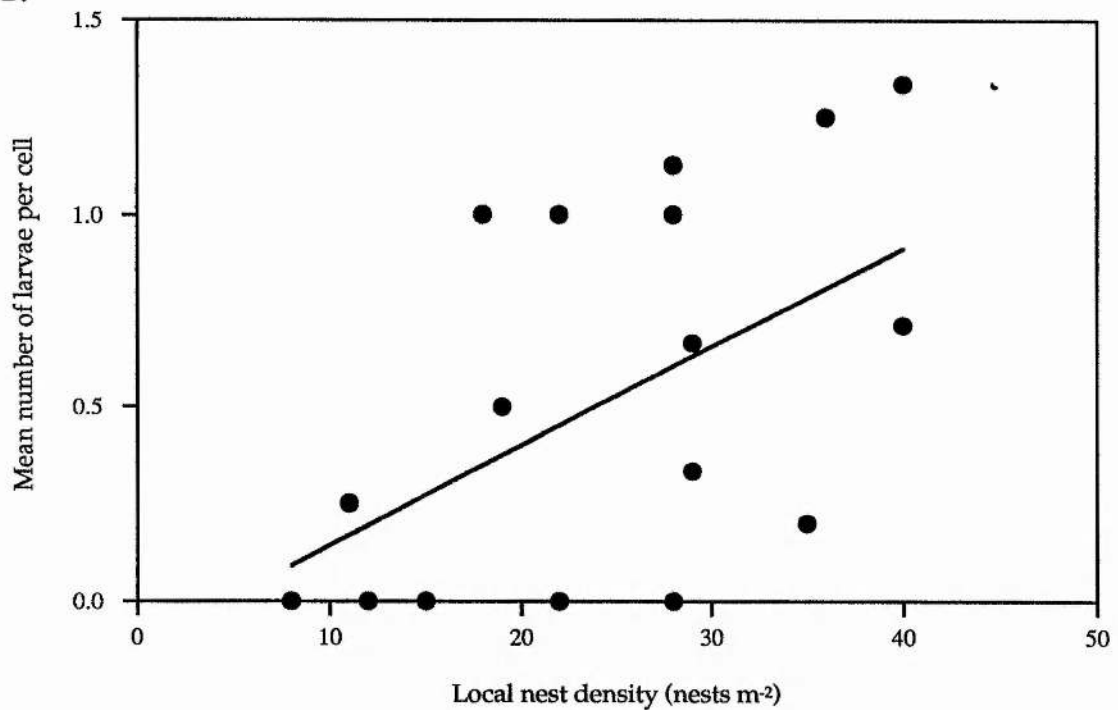
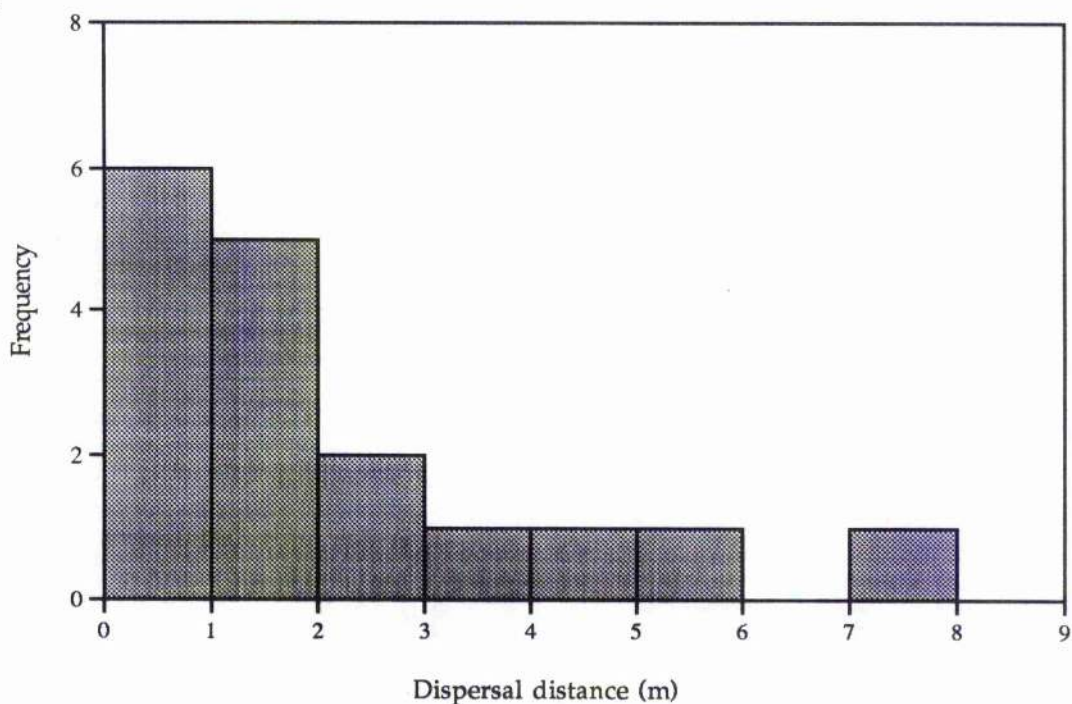


Fig 5.5. (a) Total number of diptera larvae per nest and local nest density ($y = 0.156x - 1.495$, $r^2 = 0.276$, d.f. = 18, $p = 0.017$). **(b)** Mean number of larvae per cell for each nest and local nest density ($y = 0.026x - 0.115$, $r^2 = 0.230$, d.f. = 18, $p = 0.032$). Local nest density is measured by counting the number of nests in a 1m² quadrat with the nest at the centre.

A.



B.

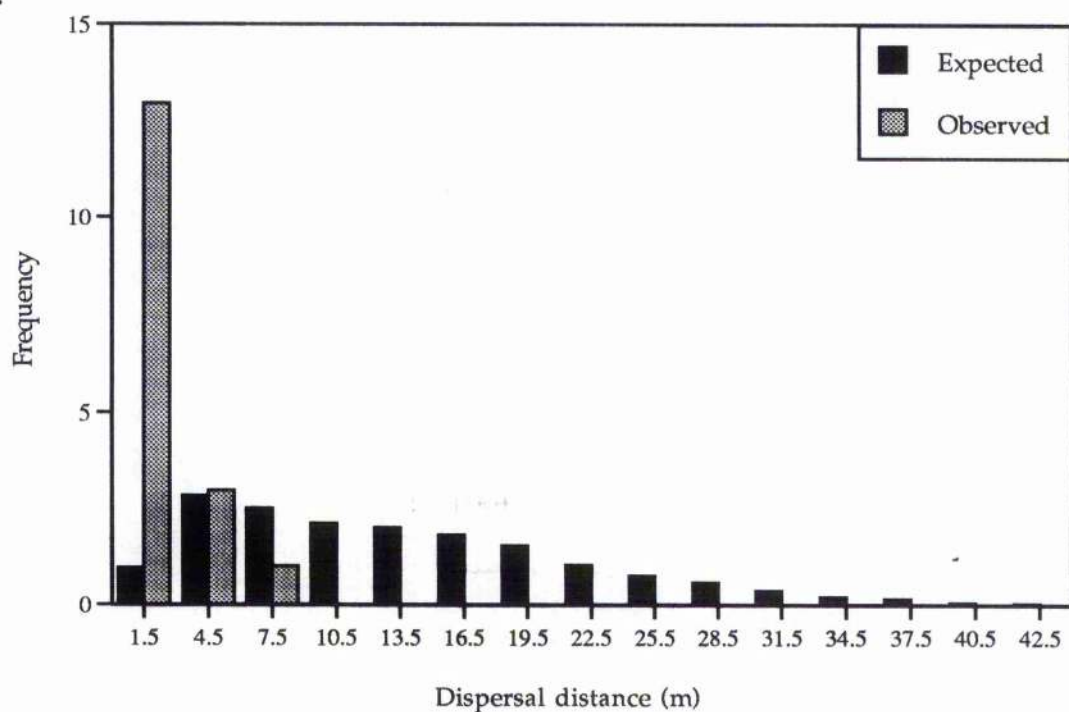
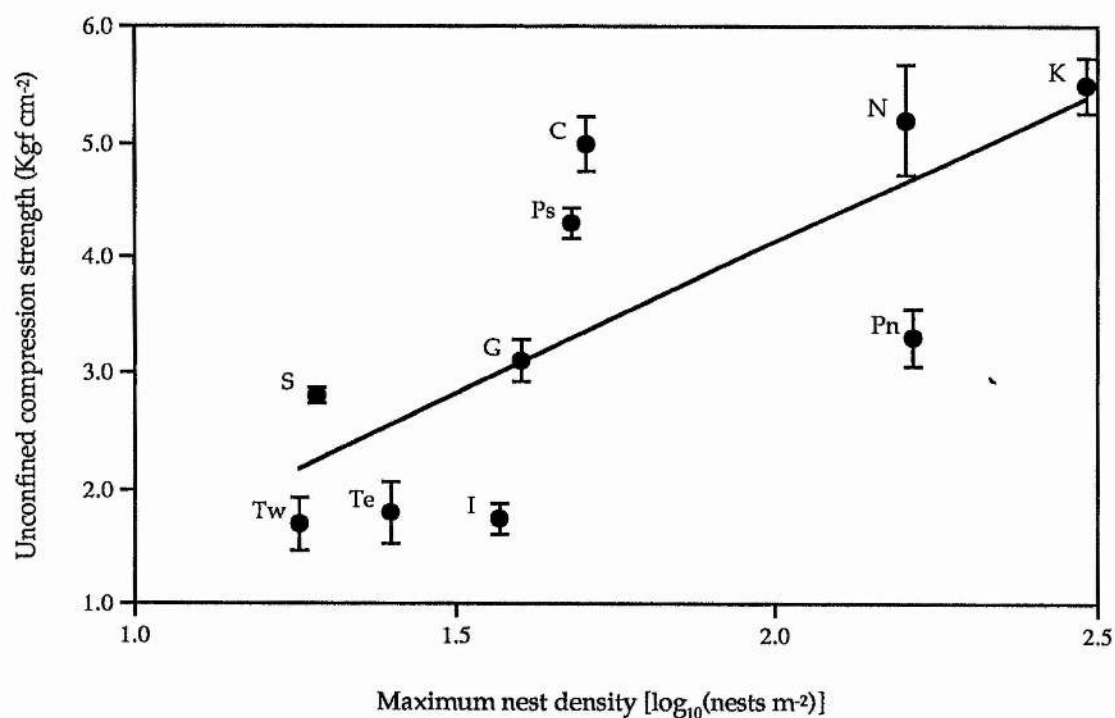


Fig 5.6. (a) Dispersal distances (distance between natal and newly founded nest) recorded for marked females at Invergowie between 1992 and 1994. **(b)** Comparison of observed and expected (computer generated random model) dispersal distances (Chi-square = 90.99, d.f = 2., $p < 0.001$)

A.



B.

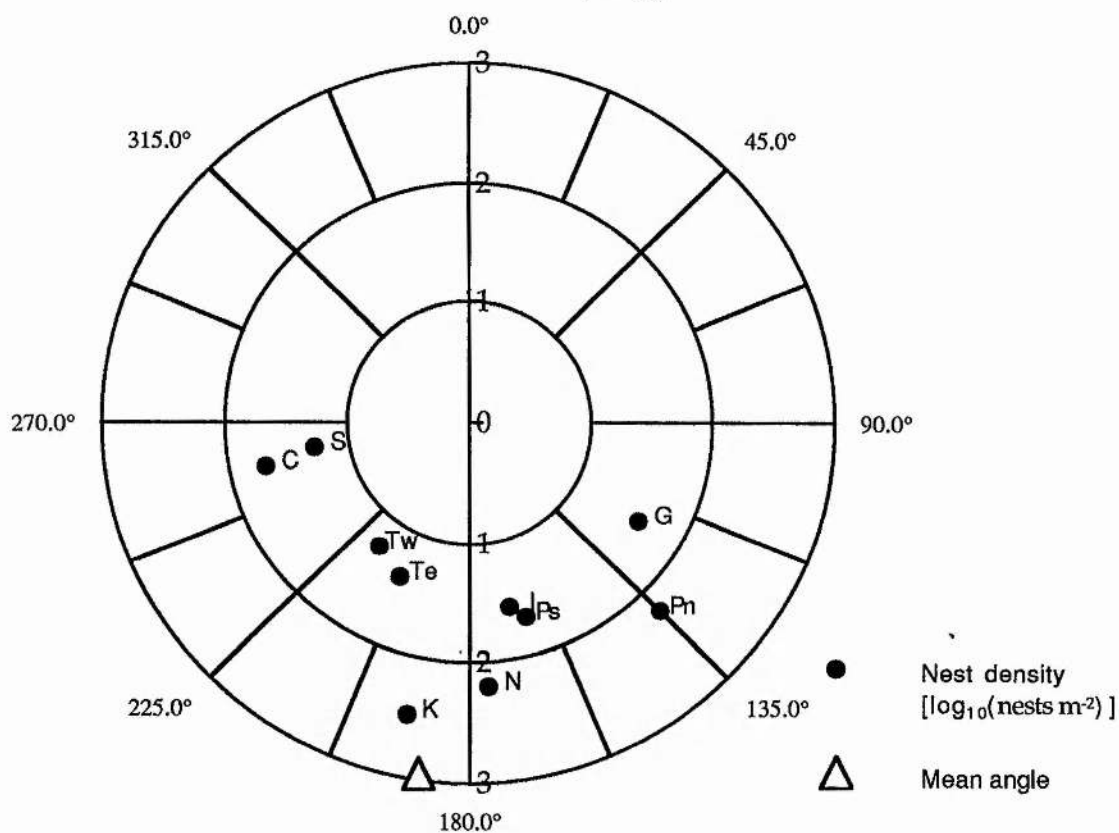
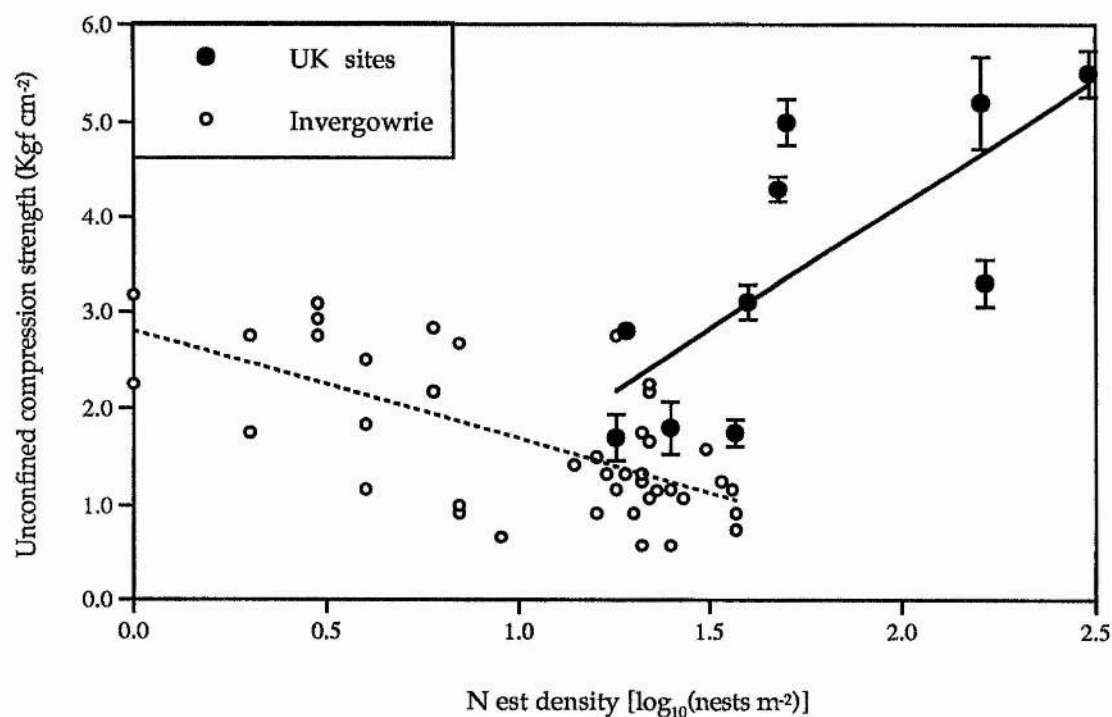


Fig 5.7. (a) Hardness and nest density ($y = 2.618x - 1.109$, $r^2 = 0.561$, d.f. = 8, $p = 0.013$). **(b)** Aspect and nest density. Magnetic north is 0°; mean angle with 95% confidence limits is $188.2 \pm 32^\circ$; V test (assuming $\mu_0 = 180^\circ$), $u = 3.224$, $n = 10$, $p < 0.0005$.

A.



B.

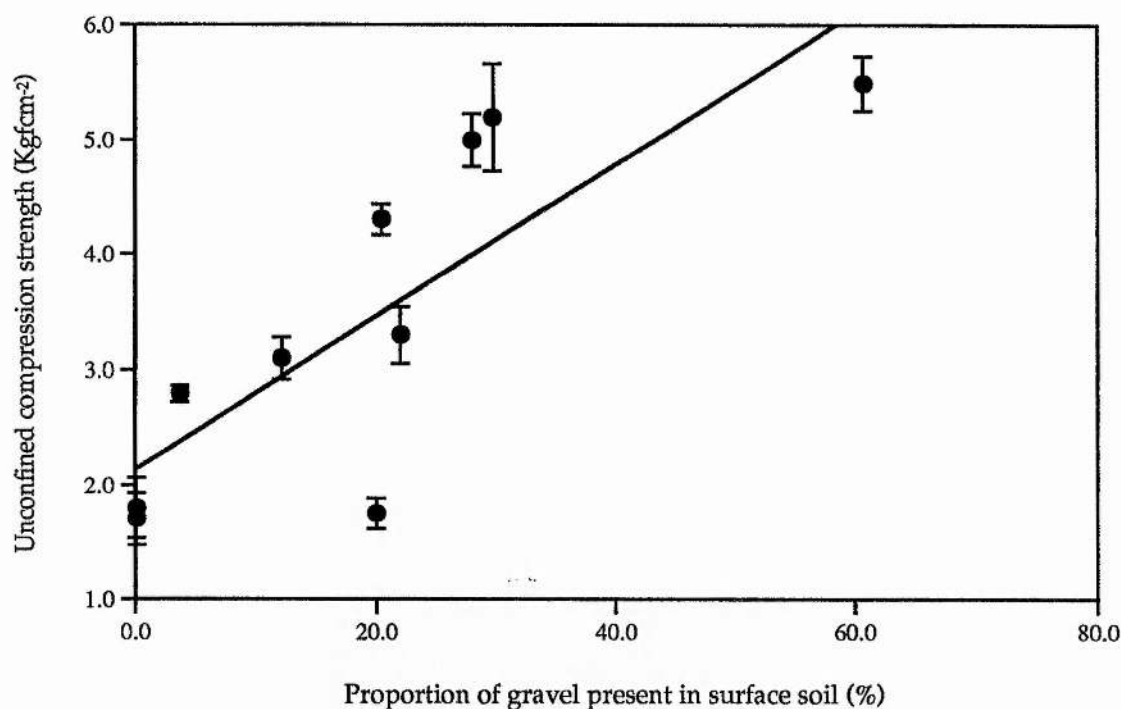


Fig 5.8. (a) Hardness and nest density for within site (Invergowrie: $y = -1.112x + 2.805$, $r^2 = 0.416$, d.f. = 40, $p = 0.000$) and between sites (UK: $y = 2.618x - 1.109$, $r^2 = 0.561$, d.f. = 8, $p = 0.013$). (b) Hardness and proportion of gravel present in surface soil for UK sites ($y = 0.066x + 2.146$, $r^2 = 0.652$, d.f. = 8, $p = 0.005$).

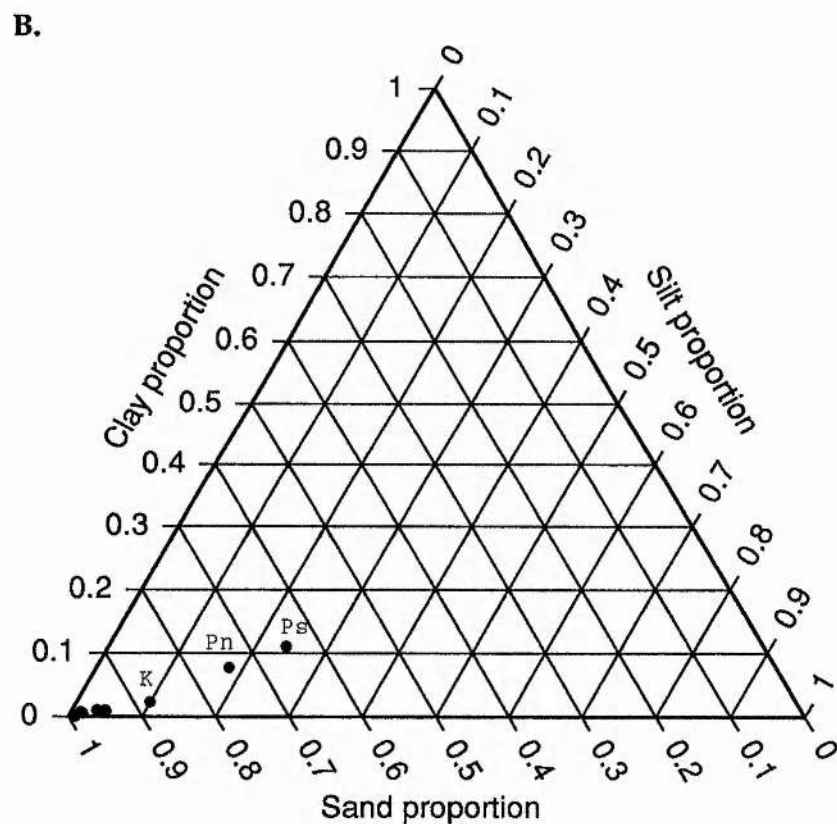
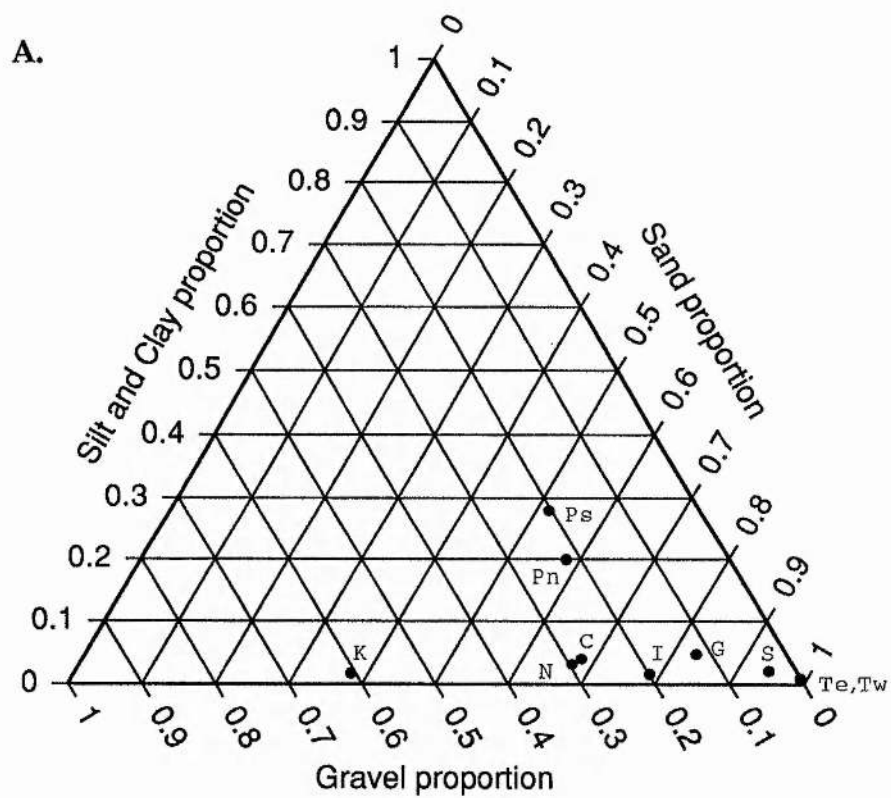
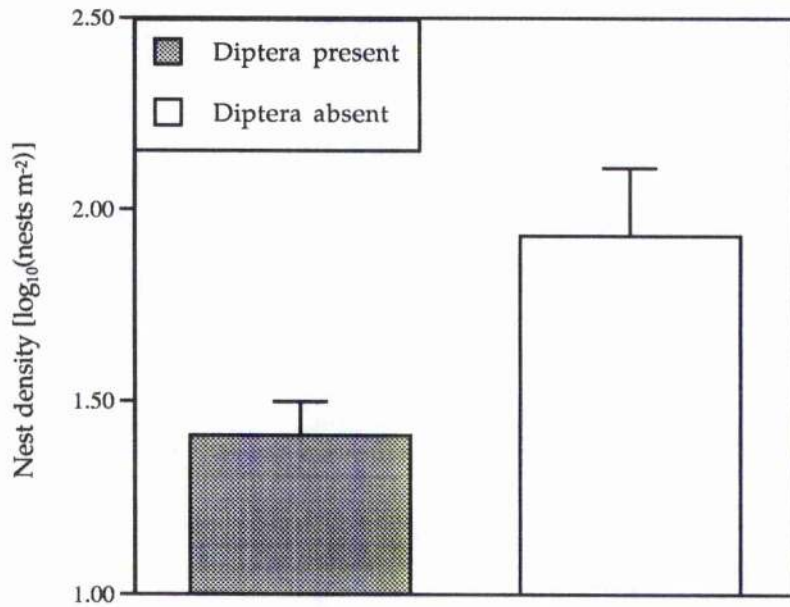


Fig 5.9. Textural classification diagrams showing relative proportions of various soil constituents found in the surface soil at various sites across the UK ($n = 10$). (a) Gravel, sand and silt & clay. (b) Sand, silt and clay. Texture classes according to the British Standard System.

A.



B.

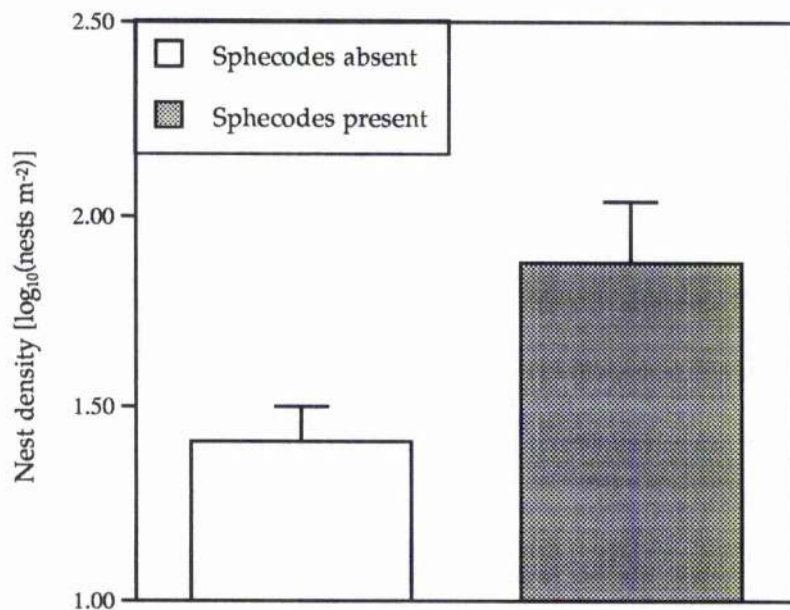
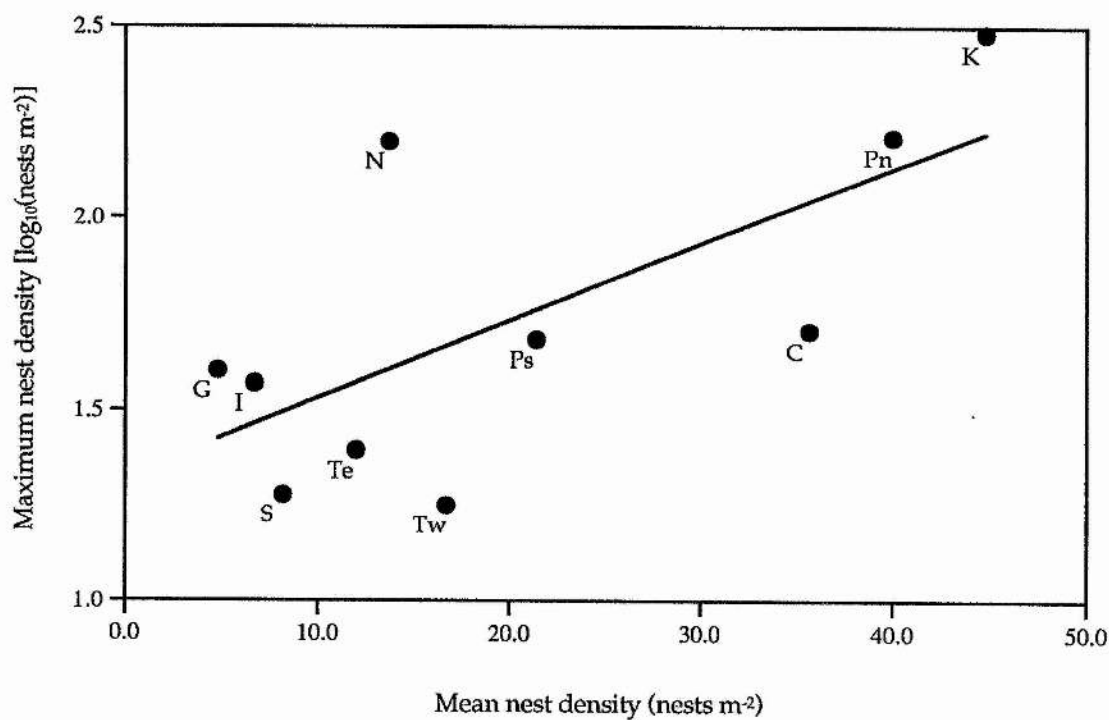


Fig 5.10. (a) Mean density of nests for aggregations where parasitic diptera are present and absent (two sample t test: $T = -2.68$, d.f. = 5, $p = 0.022$). (b) Mean density of nests for aggregations where *Sphecodes* are present and absent (two sample t test: $T = -2.55$, d.f. = 7, $p = 0.019$).

A.



B.

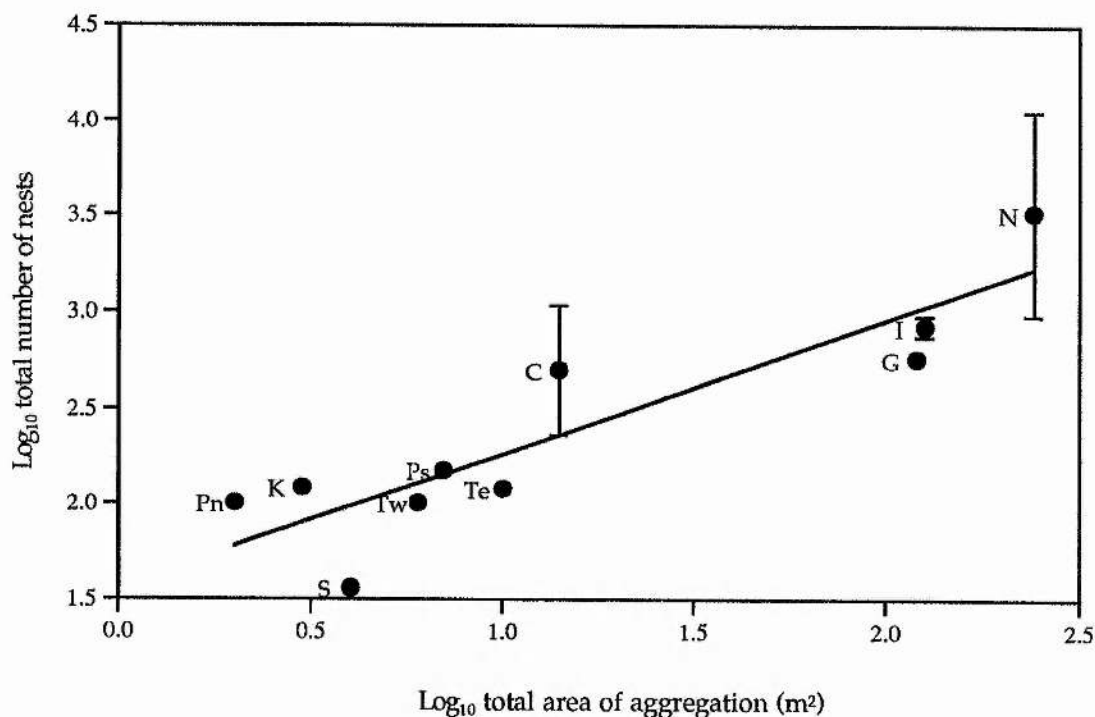
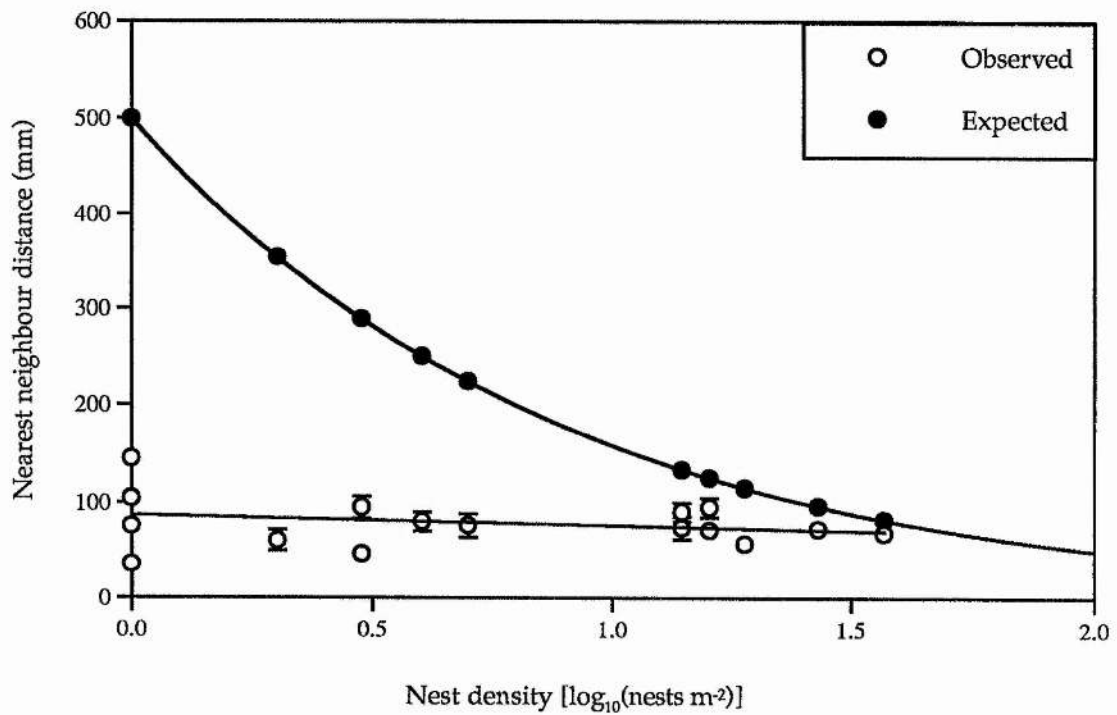


Fig 5.11. (a) Maximum and mean nest densities ($y = 0.020x + 1.331$, $r^2 = 0.479$, d.f. = 8, $p = 0.027$). (b) Total number of nests in aggregation and total area of aggregation ($y = 0.697x + 1.563$, $r^2 = 0.806$, d.f. = 8, $p < 0.001$).

A.



B.

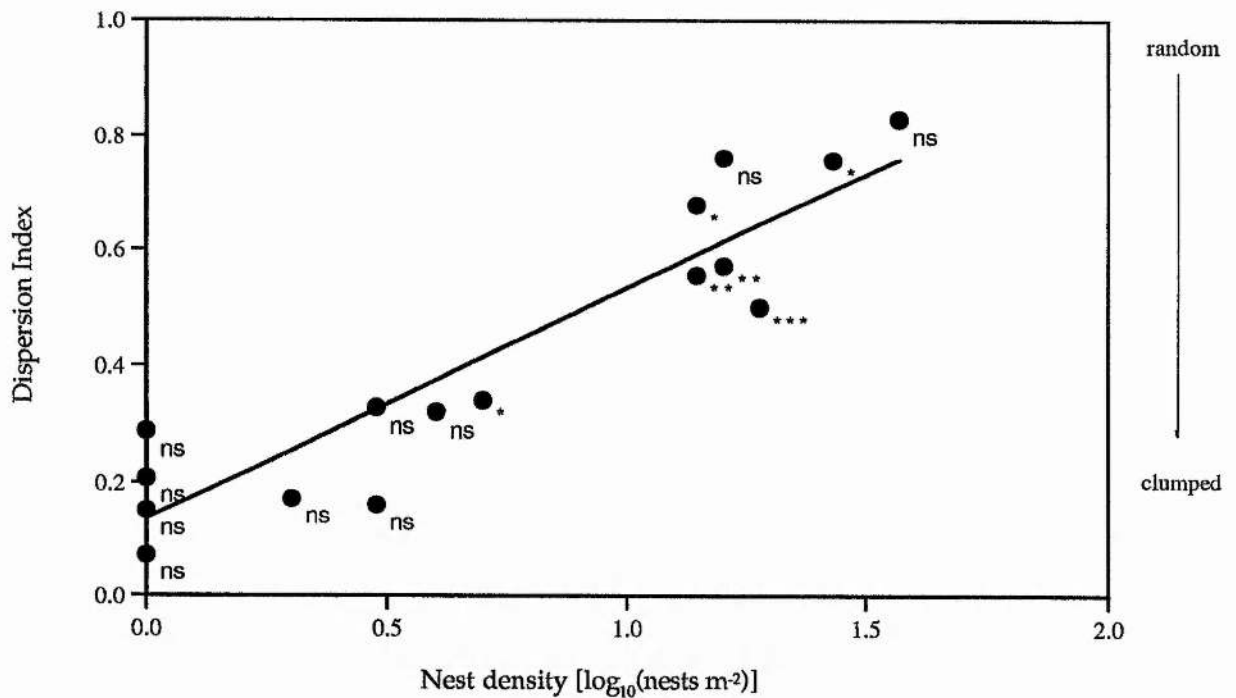


Fig. 5.12. Spatial distribution of nests at Invergowrie. (a) Nearest neighbour distances (NND) and maximum nest density, observed NND ($y = -11.009x + 87.205$, $r^2 = 0.062$, d.f. = 14, $p = 0.335$). (b) Dispersion index (ratio of observed NND to expected NND) and maximum nest density ($y = 0.396x + 0.138$, $r^2 = 0.853$, d.f. = 14, $p < 0.001$). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; 'ns' non significant.

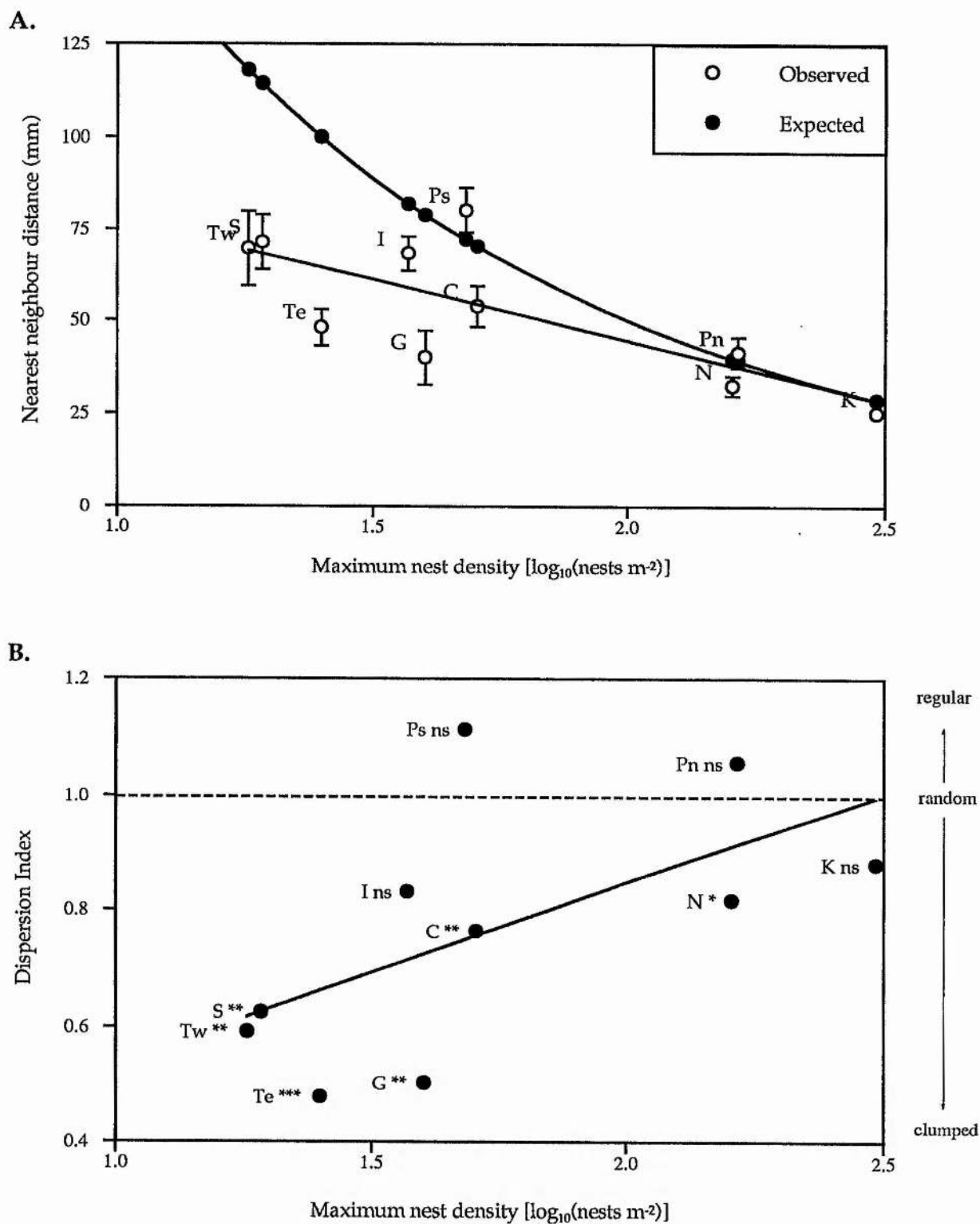


Fig. 5.13. Spatial distribution of nests for sites across the UK (a) Nearest neighbour distances (NND) and maximum nest density, observed NND ($y = -33.014x + 110.443$, $r^2 = 0.559$, d.f. = 8, $p = 0.013$). (b) Dispersion index (ratio of observed NND to expected NND) and maximum nest density ($y = 0.310x + 0.228$, $r^2 = 0.362$, d.f. = 8, $p = 0.066$).

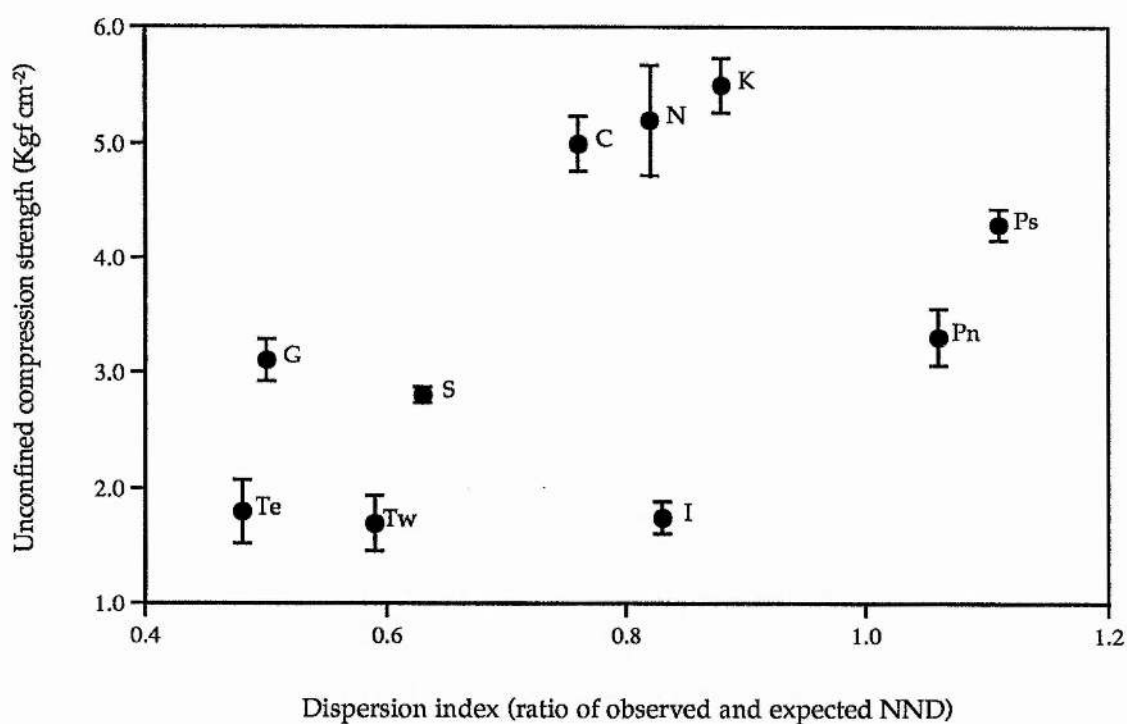
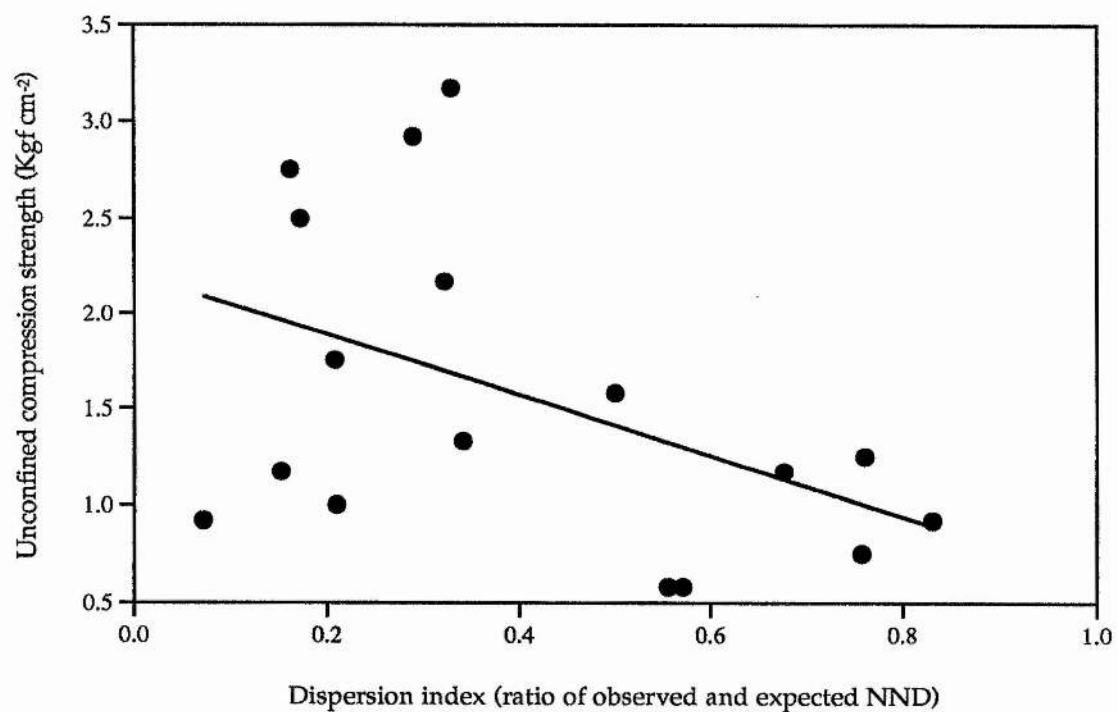


Fig 5.14. Soil hardness and dispersion index for: (a) Invergowie ($y = -1.584x + 2.204$, $r^2 = 0.213$, $p = 0.062$); (b) UK ($y = 3.326x + 0.897$, $r^2 = 0.241$, $p = 0.150$).

Chapter 6 General discussion

The preceding chapters have presented the results of three areas of investigation: the general biology, the thermal biology and nest-site selection in *H. rubicundus*. It is apparent that there is considerable overlap in these three areas, and the following discussion attempts to bring together these findings in a more unified form with reference to other works. In addition, section 6.4 demonstrates the importance of understanding both the thermal biology and nest-site selection behaviour of a bee when assessing a candidate species for possible introduction to an area. Under certain conditions, *H. rubicundus* may represent such a candidate, and the techniques used in this thesis show how other species of ground nesting bees may also be examined. The variation in sociality of halictid bees is closely linked to prevailing environmental conditions and this is discussed in section 6.5.

6.1 Discussion of some aspects of the general biology of *H. rubicundus*

6.1.1 Phenology, foraging and reproductive output

The *H. rubicundus* population at Invergowrie exhibits a life cycle characteristic of many other species of univoltine solitary ground nesting bees in temperate climes (O'Toole & Raw 1991). It is thought that many halictine species are solitary; however, few have been adequately documented (Sakagami 1980; Packer *et al.* 1989b; this study). The phenology of *H. rubicundus* is highly seasonal and the bee depends upon the warmer months of April through to September for its reproductive activities. The flight season is somewhat extended in southern UK populations where the warmer climate allows females to emerge earlier from diapause.

Fertilised females emerge from their overwintering hibernacula and initiate new nests in the spring. Like other halictine species *H. rubicundus* females are mass provisioners; a single pollen ball per cell is constructed after the collection of sufficient nectar and pollen. As a species, *H. rubicundus* is known to collect pollen from a wide range of sources (Table 3.1); and at the population level (Invergowrie) at least 14 morphologically distinct types of pollen are collected. Similarly the halictid *Lasioglossum zephyrum* was recorded collecting pollen and nectar from no less than 30 families of flowering plant (Batra 1966).

In a single day, when not restricted by poor weather conditions, a typical female *H. rubicundus* will make six foraging trips returning with pollen (and nectar?), and two without pollen (and presumably with nectar) (section 3.3). Similarly Michener & Wille (1961) found that *Lasioglossum imitatum* females made one to seven trips a day for pollen and sometimes one or two others for nectar gathering. In halictines, there is a tendency for afternoon trips to be for nectar only and it has been suggested that the nectar is probably used by the individual female (Michener 1974). However, it may also be that for *H. rubicundus* (and other halictids), that the final foraging trips of the day are used to gather nectar to add to the pollen loaf. This would be necessary to form the correct consistency for sculpting after the addition of several pollen loads earlier in the day.

A similar forage pattern to that of *H. rubicundus* at Invergowrie, was found by Free and Nuttall (1968). A variety of bees visiting flowers of *Brassica napus* collected nectar throughout the day, while pollen collection was largely restricted to the morning. The peak pollen availability at *B. napus* is assumed to be early in the day (08:00-10:00), as this is the time when honeybees collect most pollen (Williams 1985). This would appear to be the case for *H. rubicundus* (Figure 3.3); although the influence of the actual dehiscence patterns of *B. napus* at Invergowrie need further investigation. An estimate of 11 forage trips is necessary to completely

stock one cell and provide enough food for the entire development of a single offspring (section 3.5.2) so that 2 to 3 days per cell will be required. The equivalent of nearly 60 pollen collection trips in total are necessary to produce a full brood, which includes provisions lost to *Leucophora grisella* larvae (section 3.9).

The prevailing weather conditions at Invergowrie mean that females have severely restricted periods for foraging; thus the productivity per foundress is relatively lower (3.9 offspring) than it might be at other sites (e.g. Kildale 8 offspring, section 3.2) and for other solitary halictids in temperate areas (e.g. *Lasioglossum figueresi*, 5.0 offspring, Wcislo *et al.* 1993). This low productivity near the limit of a species' geographic distribution is not unusual. Packer *et al.* (1989b) observed that *Augochlorella striata* produced only 6.4 offspring per nest at the northern edge of its range; whereas under less stringent environmental conditions nests would be expected to produce many more offspring (> 13); though this may also be a result of their expanded social organisation (see section 6.5).

Productivity may also be influenced by female size within a population, as offspring size will depend upon the amount of pollen provided (e.g. *Augochlora* sp., Michener 1974). Invergowrie bees have relatively large head widths for a UK population (section 3.7.1); accordingly offspring will require a larger amount of provisions for development. It might be expected that smaller females from southern populations would need to make fewer foraging trips in order to adequately stock cells; however, they will also carry smaller pollen loads while foraging. Further data from other sites would be necessary to resolve this.

Nest architecture at Invergowrie is usual for a halictine bee (Sakagami & Michener 1962), with a vertical main burrow having several cells with short laterals connected to it (section 3.4). Nests are often sited close to stones (section 4.3.2) and the resulting elevation in nest temperature will have the advantages of allowing

quicker brood development times (Jeanne & Morgan 1992) and allowing females to warm up more quickly in their burrow entrances (section 4.5.4).

Cells are excavated and provisioned sequentially by the founding female, and the offspring begin to emerge in mid June. At Invergowrie (and presumably other UK sites) emergence is protogynous and across this site the mean number of surviving offspring is 2.2 females and 1.7 males per nest. This gives an emergent sex ratio of 1 female to 0.77 males. In contrast, Yanega (1988) found sex ratios of 3:1 in the first brood and 1:1.5 in the second brood of the primitively eusocial nests of this species in the USA. This highlights one of the major differences in social organisation, with the first brood being predominantly female (half of which remain in the nest as a worker caste) and the second brood being predominantly male. The variability of sociality within this species will be discussed further in section 6.5.

At Invergowrie the main provisioning phase is from early May to late July; the first two months of this period coincide with the local availability of *Brassica napus* pollen (section 3.5.1). *H. rubicundus* shows a definite preference for this pollen source above the broad range of other pollen hosts available in closer proximity to the nest-site. This preference is so strong that females will make a 3 km round trip to visit plantations of this flower. Wille & Orozco (1970) reported that, in Costa Rica, the small halictid *Lasioglossum umbripenne* would return to its nest when released more than 100 m away. This estimate of forage distance is considerably less than that of *H. rubicundus*; however the gross differences in habitat type means that the drawing of sensible comparisons is difficult (it is also highly questionable whether the above is an indicator of forage distance at all). Many other species of bees, both solitary and social, will travel for several kms while foraging (reviewed by Roubik 1989). Other potential pollen sources at Invergowrie were only a few 100 m distant (e.g. *Rubus idaeus*); but the incidence of

H. rubicundus on several different varieties of raspberry was very low (Willmer *et al.* 1994), even though it was one of the most locally abundant pollinators at the S.C.R.I. Some possible reasons for this are discussed in section 6.4.

During the *B. napus* flowering period female bees will still collect pollen from other sources; and after the *B. napus* has finished flowering, females continue to collect pollen from more than one source during a single forage trip (Table 3.2a). Consequently pollen balls contain a wide variety of pollen types (Table 3.2b). *H. rubicundus* must have evolved the ability to use nectar and pollen from several different flowers, in order to remain active for such a long flight season. This polylectic nature is not uncommon in other temperate halictid bees (Westrich, 1989).

6.1.2 Nesting site and parasites

The nesting aggregation at Invergowrie is a somewhat anomalous site, as it supports a massive population of parasitic Diptera. *Leucophora grisella* is a very effective nest parasite as 65.0 % of all nests had a least one *L. grisella* larva present, and in these nests 29.1 % of the cells had been attacked (section 3.8.1). The association of *Leucophora* species with halictid bees is well known (e.g. Knerer & Atwood 1967; Roberts 1973); but the level of brood mortality is commonly much lower (e.g. 10 %, Batra 1965; 0.3 % Packer *et al.* 1989a). Two suggestions can be forwarded to explain the differences between these earlier works and the present study. Firstly, the species observed in other studies had primitively eusocial nests, which may reduce the incidence of parasite entry to the nest (see section 6.5). Secondly the Invergowrie population may be exhibiting the maximum brood mortality likely to be observable, as the aggregation is in fact dying off. As a bee aggregation becomes established it takes some time before a population of parasites reaches sufficient numbers to cause a significant amount of brood

mortality, and it is likely that the two studies cited above have recorded this stage in the aggregations history. Later when the parasites are inflicting much higher mortality the bee population begins to collapse, and this is the terminal stage that Invergowrie has reached in 1995. The quadrat of highest density of this site sustained a decrease in nest numbers of 37 to 12 between 1994 and 1995, and the overall abundance of flying bees at the site was also greatly reduced (pers. obs.). Whether the aggregation will be destroyed completely, or will recover as the parasite population collapses, due to the lack of hosts, is not yet known.

The aggregation at Invergowrie was presumably founded by a few fertilised females after 1976 (when the banking was built) and grew through the philopatric behaviour of overwintering females (section 5.2.6). A large number of departing fertilised females from one season were not seen in the next. Certainly many of these will have failed to make it through the winter, but some will presumably have moved away in an attempt to colonise other sites (Yanega 1990). This imperfect philopatric tendency has great adaptive value as new sites can be colonised; furthermore when an established colony is collapsing some of the females daughters will have greater chances of survival by dispersing away from their natal site. This strategy is likely to be universal in ground nesting Hymenoptera as aggregations are often founded in highly disturbed areas where a suitable nest-site will only be available for a few years.

Aggregations formed by returning to the natal site are likely to grow as more progeny are produced and nest in the same place (providing there is space and suitable substrate). Several studies have reported the extinction of bee aggregations through the impact of parasites: *Lasioglossum versatum* by a mutillid wasp and *Lasioglossum versatum* by *Paralictus* sp. (Michener 1974); *Nomia melanderi* by Bombyliidae (Bohart *et al.* 1960).

The impact of parasitic diptera at Tentsmuir is unknown; but as they were rare it is probable that the aggregation is in an earlier stage of development. The density-dependent nature of brood mortality at Invergowrie is discussed in section 6.3 with reference to nest spacing.

6.1.3 Individual body size

H. rubicundus females show a large variation in head widths across the UK (from a mean of 2.00 mm at Gibraltar point to a mean of 2.91 mm at Kildale). The variation in size can not be simply explained by variation in latitude. Latitude alone fails to take into account other factors such as altitude and maritime effects that modify microclimates. It is then more prudent to look at the ecophysiological factors that are likely to select directly for body size, especially if these data are easily collected. In this study 63 % of size variation across the UK is explained by variation in the local thermal regime (mean monthly minimum temperatures through the season, section 3.7.2). Size, heat exchange and behavioural patterns clearly interact in the biology of halictids.

Bergmann's rule has been applied to a number of groups (e.g. 'poikilothermic' vertebrates, Lindsey (1966) and birds, James (1970)). To date, however few studies have looked at latitudinal gradients in body size of insects, and more particularly Hymenoptera. Daly *et al.* (1991) found that there was an overall decrease in the body size in feral colonies of *Apis mellifera* from high to low geographic latitudes in California. In northern Europe, an analysis of formicine and myrmicine ant species showed that the body sizes of workers were positively correlated with latitude (Cushman *et al.* 1993). Both of these observations are in accordance with predictions from Bergmann's rule, although no explanations are given in the first study. In contrast, butterflies generally increase in body size into the tropics of the southern hemisphere (Barlow, 1994); but Hawkins and Lawton (1995) found that

this pattern is weak and largely reflects the replacement of small-bodied families by large-bodied families. Furthermore, in the northern hemisphere body sizes are independent of latitude, or become slightly smaller towards the tropics. These authors concluded that insufficient data were yet available to explain these patterns in terms of ecological or biological correlates.

No evidence was found that bee body sizes show strong or consistent relationships with latitude in the eastern US (Hawkins 1995). Several families were examined, and there was no latitudinal gradient in body size when all eight families were grouped together. Within five of the families (Colletidae, Halictidae, Xylocopidae, Apidae and Megachilidae) there was no relationship between these two variables; however, within the Andrenidae there was a positive correlation of size with latitude and in contrast a negative correlation within the Melittidae and Anthophoridae families.

In Papua New Guinea, the body mass of the bee *Amegilla sapiens* was found to increase with altitude (Stone 1993b). This was interpreted as a response to lower ambient temperatures encountered at higher altitudes, in accordance with Bergmann's rule. In addition high altitude populations were recorded as having higher T_{thS} , once the effect of body mass had been controlled for; this being suggestive of further morphological and/or physiological adaptations to lower T_{aS} .

Ghazoul (1993) showed that, in the sphecoid wasp *Mellinus arvensis*, 36 % of the variation in size was due to latitude, and 41 % was explained by soil hardness. The latter is important as it is correlated with the value of a nest; and larger individuals within a population have greater success in aggressive encounters while defending or attempting to usurp a nest. It was proposed that large size is

selected for at sites with compact ground and this environmental gradient is the most important factor in explaining wasp size.

There is then no general pattern to be drawn from this and it is assumed, at the family level at least, that latitude is not a useful predictor of body size for bees. Its influence is presumably too coarse grained and is masked by other ecophysical gradients. Indeed there is such an array of variation in other ecological and behavioural characteristics that influence body size for each species (and population) within each family, that it would seem unlikely that any particular gradient could be found that ultimately explains the observed patterns.

The variation in body size within a species is relatively easy to explain when the behavioural ecology is well understood (e.g. Ghazoul (1993); Stone 1993b, this study) However, at higher taxonomic levels the number of possible ecological correlates that could potentially explain biogeographical patterns is enormous and general conclusions are often hard to draw. Therefore, undoubtedly the thermal biology and ecology of insects will be significant determinants, if not the primary determinants, of size gradients. The choice of the size character used in any investigation of this type is of crucial importance, as it must be a direct measure of (or highly correlated with) some morphologically relevant feature that represents a physiological adaptation to temperature (Scholander, 1955). Insect head and body sizes are sensible measurements (Cushman *et al.* 1993; Hawkins 1995), but wing length/span may not be such a good index of body size (e.g. Barlow 1994; Hawkins & Lawton 1995).

6.2 Discussion of thermal biology of *H. rubicundus*

6.2.1 Thermal physiology

H. rubicundus is a typical behavioural thermoregulator and apparently not capable of endothermic heat generation, except for the obligate metabolic heat produced during flight. When live individuals warmed up in the laboratory they never reached a temperature above that of ambient (section 4.2.2); additionally the cooling constants for live and dead individuals of a given mass were identical. In the field the T_{th} on T_a regression did not have a slope significantly different from 1 (4.5.4.A). All three of these observations indicate a lack of endothermy. Many of the Apoidea families containing members with body masses greater than those of *H. rubicundus* have been investigated, and it has been shown that they are able to warm up actively before flight in the absence of alternative heat sources (see section 1.1 for examples). Endothermy is not entirely ruled out in *H. rubicundus*, as bees of similar size (and smaller) have been shown to warm up actively (Stone & Willmer 1989b). The very small (10 mg) halictid, *Lasioglossum smeathmanellum*, had a mean warming rate of $1.25\text{ }^{\circ}\text{C min}^{-1}$; this is however much smaller than any other rates recorded. Presumably in the field (with wind effects) such a small rate would have little noticeable effect on the body temperature of this species.

Herrera (1995) investigated the thermal biology of the solitary bee *Andrena bicolor* in Spain. In this study the females had a mean mass of $29.1 \pm 7.8\text{ mg}$ (17) which is very similar to that of *H. rubicundus* females, and the cooling constants for both dead and live bees were between 1.074 and $1.188\text{ }^{\circ}\text{C min}^{-1}\text{ }^{\circ}\text{C}^{-1}$ (calculated from the data in Table 2); no data about the masses of the six individuals tested were given by the author. It was concluded from the analysis of k values that there was no difference between live bees and dead bees; and thus *A. bicolor* (like *H. rubicundus*) was unable to generate a T_{ex} spontaneously during passive warming.

Stone (1989) suggested that all bees have some endothermic ability; this was clearly demonstrated for a taxonomically wide distribution of species showing pre-flight warm-up in the laboratory. Furthermore it was proposed that above a minimum body mass of perhaps 30 to 40 mg, bees would have varying endothermic abilities. This may well be the case as there is considerable variation in mean warm-up rates in the 50 to 90 mg mass range (1.0 to 10.5 $^{\circ}\text{C min}^{-1}$). It may be that at 36 mg *H. rubicundus* females have a warm-up rate close to zero (as does *Andrena bicolor*), while *Colletes daviesanus* (36 mg) was recorded with a rate of 3.8 $^{\circ}\text{C min}^{-1}$ (all are UK species).

The two halictids (*Nomia* sp.) of similar mass to *H. rubicundus* (listed in table 1. of Stone & Willmer (1989)), do not have rates quoted and presumably were not tested or did not show any endothermic activity. If the latter, then perhaps certain members of the Nomiinae in addition to members of the Halictinae are incapable of spontaneous endothermy.

From this, the ability of *H. rubicundus* to control its body temperature appears to be purely dependent upon behavioural strategies. Basking on the ground by both sexes has been shown to be an effective mechanism for elevating T_{th} (section 4.5.3). The T_{ex} achieved depends upon the size of the individual (section 4.5.4.B) and the ambient thermal conditions (section 4.5.4.A). Size profoundly influences the rates of heat exchange; the cooling constants, initial warming rates and cooling rates across the species size range were all found to be inversely proportional to size, as predicted by May (1976). There were no differences found in the cooling constants for males and females of the same size.

The flight temperatures of both males and females were highly predictable using T_a and head width as predictors (section 4.5.4.D). However, head width was only

really important at lower T_a , where its influence on whether an individual could attain a suitable T_{th} for flight was especially strong. Similarly ground temperature and head width were powerful predictors of T_{th} for baskers. Males had an average T_{th} in flight of 25.8 °C which was substantially lower than that of females (29.4 °C), and this is accounted for by the relative increase in convective heat loss with smaller size. As was expected larger females were the first individuals to leave their nests in the early morning as they were the only individuals that could maintain an elevated T_{th} necessary for flight. The T_{th} values for *H. rubicundus* are very similar to two *Nomia* species (masses 43 and 45 mg) which had T_{th} s in flight of 28.5 and 30.0 °C (Stone and Willmer 1989b). Herrera (1992) showed that the T_{th} of the hawkmoth *Macroglossum stellatarum* remained within narrow limits during flight (39 to 46 °C); and variation in T_a and L were used to predict T_{th} across a range of environmental conditions. However in this study the importance of size was ignored.

The minimum T_a for flight (MTFF) for *H. rubicundus* males was 15.5 °C and was 1.0 °C higher than that for females. These values are considerably less than the 20 °C MTFF recorded for the *Nomia* species referred to above. This is explained by the *H. rubicundus* population at Invergowrie being a cool temperature species and the *Nomia* spp being of tropical origin (Papua New Guinea). As enzyme systems are adapted to the usual environmental conditions experienced by a species and possibly a population (Heinrich 1981), it would be expected that the MTFF for *H. rubicundus* would be much lower than for the *Nomia* spp.

Upon initiation of flight after basking, males experience a decrease in T_{ex} of 1.3 °C whereas females warm up by some 3.3 °C (section 4.5.3). Males and females then are on opposite sides of the size boundary where the increase in metabolic heat production during flight is equal to the increased convective heat loss. Therefore

the microclimatic window within which males can fly is more restricted than that for females.

6.2.2 Temperature, behaviour and abundance

The relative abundances of bees were predictable from the two microclimatic variables of L and T_a . Predictions within a single day were highly accurate (section 4.4.2); however, those made using data across a season were less powerful but still useful (section 4.4.3). Extensive data are required to produce such a model, and T_{so} s were tested as a more convenient method of predicting abundance, but were found to be much less effective (section 4.4.3). Only 20.1 % of female abundance was accounted for by variation in L , and T_a and time of day were found not to be important (although these are all related (section 4.3.3)). This was probably a short-coming of the linear regression model used rather than a real phenomenon, and it would be expected that considerably more variation than this could be explained by microclimate. For males, however, 75.0 % of the abundance variation was attributed to L and T_a .

Other studies have found that T_{so} s were very good predictors of various behavioural patterns, but these tended to be restricted to observations made within a single day. Burrill & Dietz (1981) found that the departure rate of honey bees from a hive was proportional to L up to 0.66 Langleys and then inversely proportional above that level; the response to T_a was positive and linear. No explanation of the change in form of the abundance relationship was given, but presumably there was a response to overheating which would be likely at high L and T_a values. Corbet *et al.* (1993) showed that the abundances of flying *Apis mellifera* and several *Bombus* sp. were highly predictable from black globe temperatures (a function of T_a and L). Flight activity increased proportionally with globe temperature up to a species specific threshold, and was then

independent of globe temperature above this value. The diurnal activity of *Bembix rostrata* in the field was related to T_a , L and cloud cover (Schöne & Tengö 1992). Flight activity was positively correlated with both T_a and L , with T_a having the highest overall r^2 values for the regressions. At an insolation change, activity levels responded almost immediately (lag < 1 min) before the resulting change in T_a was realised. It can be concluded that T_a is a good general determinant of activity through a day, but microtemporal responses are largely accounted for by fluctuations in L . All three of these studies give useful accounts of flight activity changes through a day of given microclimatic conditions; but their applicability was not tested across a wider range of conditions.

Louw & Nicolson (1983) were able to determine the minimum flight temperature of the carpenter bee *Xylocopa capitata*, across several days, as a globe temperature of $\approx 23^\circ\text{C}$. Above this T_{so} the number of bees observed in foraging and territorial activity increased proportionally with the globe temperature. Similarly the percentage of *Thymelicus lineola* (Lepidoptera) individuals feeding, flying and courting (males only) was positively correlated with black globe temperature (Pivnick & McNeil 1987). This study used data from two summers in Canada, and effectively described the proportion of a population undertaking a particular activity using T_{so} s.

The diurnal abundance of *H. rubicundus* is clearly unimodal (section 4.4.2). There is a huge literature dealing with the factors influencing the modality of bee behavioural patterns. In general, bee activity patterns may be determined by microclimatic limitations such as temperature (e.g. *Andrena erythronii*, Michener & Rettenmeyer 1956; *Dialictus umbripennis*, Eickwort & Eickwort 1971) and/or changes in floral resources (e.g. *Andrena omninigra clarkiae*, MacSwain *et al.* 1973; *Bombus* sp., Willmer 1983). Within a given day there will be definite microclimatic and floral resource windows which absolutely limit bee foraging (see also section

6.4). All other things being equal, it would be expected that females would exploit the overlap in these two windows maximally in order to provision as many cells as possible. However, several other factors will modify this ideal pattern: competitive exclusion by other foraging species, nest cell cycle (e.g. a limit of one cell per day) and mating requirements. Detailed knowledge of both the thermal biology and foraging behaviour of a species is necessary in order to determine the primary cause(s) of abundance patterns.

H. rubicundus females commence foraging as soon as T_a exceeds MTFF in the morning (4.5.4.E); this is always after flower opening during the *B. napus* season, so the pattern is initially temperature dependent. This species would be able to forage much more effectively through the season if it was capable of endothermy and continual flight at much lower T_a s. This would be of great advantage at the cooler sites, such as Invergowrie, where adverse weather means that many days in the season fail to reach MTFF. Females cease foraging when T_a is still greater than the MTFF in the afternoon of many days; this would indicate that some other factor brings about the end of flight activity. This cannot be the need to excavate a new cell once one is complete, as a cell takes at least two days to fully provision. Nest foundresses are mated during the season before nest construction, and so mating need is ruled out. Also it is unlikely that pollen source(s) are not available, as *B. napus* flowers remain open well into the evening (section 4.5.2), and *H. rubicundus* is capable of using a whole range of other sources. One possibility explaining the afternoon cessation is that the rewards at the *B. napus* plantations are too small for a 3 km trip to be worthwhile in terms of energetics. It may also be that the nectar becomes too concentrated in the afternoon and so is unavailable to *H. rubicundus* females. It appears, then, that the decline in activity is probably floral resource limited. Further investigations at the forage sites of *H. rubicundus* will be necessary to confirm this, and these will have to be detailed enough to disentangle thermal and resource limitations as the quantity and quality of floral

rewards are of course determined to a large extent by microclimate (Corbet *et al.* 1979).

The primary motivation for *H. rubicundus* males to fly is mating opportunities. Presumably males attempt to encounter females as often as possible; and as MTFF for males is less than that of females, males are forced to walk on the banking when females are just able to fly at lower T_{as} . The thermal window for male flight activity is smaller than that for females; but through walking males manage to maximise their mating potential and so the abundances of the two sexes are highly correlated. The initiation of male activity is directly temperature dependent and the cessation is probably directly dependent upon female activity, and hence indirectly dependent on the availability of floral resources.

There may also be sensory determinants in flight activity patterns; it is still unclear whether sun irradiance, in addition to physically warming an insects body, has an effect in stimulating flight activity via the CNS (or humoral system?) (Schöne & Tengö 1992). Certainly many daily activity patterns of animals have underlying internal rhythms regulated by light levels (McFarland, 1985). This has not yet been examined for the activity patterns of solitary bees.

The lack of endothermy may possibly limit the geographic range of *H. rubicundus*, as an ability to elevate T_{th} prior to flight would decrease MTFF and so allow females to forage with less microclimatic restrictions. The population at Invergowrie is already fairly close to the northern limit of the species range, although it can be found in the central Scottish Highlands (Else, in prep.). The very closely related halictid, *H. tumulorum*, is distributed across the UK but its northern limit is the Scottish Borders. This bee is much smaller (9 - 17 mg) than *H. rubicundus* and so presumably is even more subject to restrictive thermal considerations. Having an even smaller thermal window for foraging suggests

that it would be unable to survive at the northern extremes of the distribution of *H. rubicundus*.

The variation in size of *H. rubicundus* across the UK was discussed in section 6.1. The thermal advantage of a large body size is reduced heat loss (section 4.1), which results in an ability to forage (females) and seek mating opportunities (males) at lower T_a s. Larger males would have a mating advantage as they are able to pursue females at lower temperatures when smaller males are unable to fly, much as Stone *et al.* (1995) found with *Anthophora plumipes*. However there is a cost in reproductive success associated with this. As large offspring require more provisions, inevitably populations of large individuals will produce fewer offspring overall, and this is the case at the limit of the *H. rubicundus* range (section 6.1). Furthermore, fewer offspring limit the type of social organisation that can be achieved within a nest (see section 6.5).

6.3 Discussion of Nest-site selection of *H. rubicundus*

Chapter 5 examined a wide range of abiotic and biotic factors that influence nest-site selection in hypogeous Hymenoptera. Several factors were found to be closely associated with the nest density of *H. rubicundus*, both within (section 5.2) and across (section 5.3) sites. The spacing of nests within an aggregation was also found to be influenced by various characteristics of the nesting site (section 5.4).

For various properties of the substrate (Table 5.6), the range of values where nests are found may be very narrow (e.g. soil particle size) or very broad (slope). Nest-site quality will be dependent upon an array of these factors, many of which will be closely related. In many cases *H. rubicundus* may be able to tolerate sub-optimal conditions of one factor providing that other factors are highly suitable.

Many of the properties analysed may be of little ecological relevance on their own, but together with several other factors may contribute to the quality of one primary (ultimate) factor determining site preference. For instance nest temperature will be an important component in the productivity of a nest (Jeanne & Morgan 1992; see previous discussion). Nest (and ground) temperature is a product of at least five other proximal factors that determine the amount of solar radiation that the ground receives. Latitude and altitude will determine the intensity and duration of sunlight to which a piece of ground is subjected. Slope, aspect and soil colour will determine the amount of available radiation that is then absorbed. As it is difficult to measure ground temperature meaningfully across a number of sites, the measurement of proximal cues will be useful in estimating the ultimate value of a site.

Whether a searching female uses the ultimate factor as a cue or some proxy of it, is not clear from this study. Hymenoptera are certainly able to perceive visual (Wcislo, 1992), olfactory (Bell, 1974) and thermal (Brockmann 1979) characteristics of the environment. There are indications that females are sampling different parts of the substrate within a nest-site through antennal tapping, test digging and basking (section 5.1.2). Bees that show an ability to differentiate between areas of varying quality will have greater reproductive successes and so will be selected for.

Within the site at Invergowrie there were preferences demonstrated for the following characteristics:

- Softer soils (section 5.2.1).
- More steeply angled ground (section 5.2.2).
- Warmer soil, both on the surface and at depth (section 5.2.3)
- Sandy soils with high humidities but not waterlogged (section 5.2.4)

- Presence of stones and rocks on the surface (section 5.2.4).
- Areas with low abundances of parasitic Diptera (section 5.2.5).
- Areas close to the natal nest (section 5.2.6).

General preferences exhibited by *H. rubicundus* across UK sites are:

- Soft soils in low density aggregations and hard soils in high density aggregations (sections 5.3.1 and 5.4.3).
- Slopes with southern aspects (section 5.3.2).
- Sandy soils with high humidities but which are not waterlogged (5.3.3).
- Sites where parasitic Diptera are absent (section 5.3.4).

Many of the above preferences are documented for other Hymenoptera species (reviewed in section 1.2). None of these studies (except Brockmann, 1979) really addresses the full range of possibilities that may influence nest-site selection; and it is believed that many of the studies may have missed important determinants of nesting behaviour. The majority of studies were based on single site observations, and consequently may have overlooked the possibility that the form of a relationship between nest density and a given factor may change between different density levels. This is the case for *H. rubicundus* when soil hardness is considered. Females prefer to nest in areas of soft soil as this is energetically more economical for digging; however, at higher densities the relationship changes and harder soils are preferred as these help maintain the structural integrity of a nest when the risk of collapse due adjacent nests is high.

There is good evidence that the high nest density at Invergowrie and the other sites is attributable to the 'limited substrate' hypothesis. This is further reinforced by the philopatric tendencies of overwintering females as they return to their natal nest area to found new nests (section 5.2.6). Although this process may be

independent of the 'limited substrate' hypothesis which facilitates aggregation formation, any factor modulating reproductive rates in different nesting areas will accelerate the formation of aggregations under philopatry (Rosenheim 1990). This strategy has the advantage of reducing the search time necessary when seeking a suitable site for nest initiation; however, at Invergowrie this results in nests being founded in a location where brood mortality due to parasitic Diptera is extremely high. Presumably, as a long term strategy, philopatry has been selected for as it will increase the reproductive success of the foundresses. This must then outweigh the loss in productivity due to a brood parasite, such as *Leucophora grisella*, in the short term. Finally there is also the possibility that returning females are able to assess the abundance of flies at a site and then elect to nest elsewhere. There would be considerable advantage in doing this as the parasitic toll at a 'mature' site can be very severe. From this study, there is no direct evidence for this, though it still represents an interesting prospect.

As the incidence of parasitic larvae is density dependent (section 5.3.4), it would be expected that aggregations of lower nest density or non-colonial nesting would be favoured (Section 1.1). However, if the levels of parasitism at Invergowrie are an unusual occurrence for a population of *H. rubicundus*, then dense assemblages will still be selected for by other factors. None of the other UK sites had the same parasite abundance as Invergowrie; this may be explained by differing stages in parasite-host cycles at other sites. There may also be considerably fewer problems for southern UK populations as the primitively eusocial lifestyle that exists there may be very effective in reducing parasitic attack (see section 6.5).

The spatial positioning of nests within an aggregation may be influenced by a number of factors (Table 5.1) and these are discussed with reference to other works in section 5.4.1. At Invergowrie it was concluded that the tendency for nests to be clumped together was a result of very fine grained variations in the

substrate. However within the clumps there is more regular spacing of the nests which was attributed to the necessity of maximal nest spacing at the highest densities in order to maintain architectural stability (section 5.4.2). Across the UK, sites with the lowest densities of nests also showed clumping and it was suggested that this was again due to microscale variation in substrate quality (5.4.3). Sites with high densities exhibited random spacing; this was probably due to the net effects of limited substrate (favouring clumping) and a need to maintain structural integrity (favouring regular spacing).

6.4 Introduction of novel bee and crop species

Intensive farming methods have meant that recently it has become necessary to introduce large numbers of bees into some agricultural areas in order to achieve satisfactory crop pollination. This has arisen from: the use of vast unbroken tracts of cross pollinated crops (creating local pollinator shortages); the application of large quantities of pesticides and herbicides; and the destruction of nesting habitats (Torchio 1991).

For many years honeybees were the principle pollinators of commercial crops; however the rapid spread of tracheal and *Varroa* mites throughout the world and the establishment of the Africanised honeybees in the New World have lead to a decline in the usefulness of this species (Parker *et al.* 1987). In addition honeybees are often not the best adapted species to pollinate a particular crop efficiently; and it is often local species of solitary bees that are of greatest use (Batra 1992).

In the United states, alfalfa and its products were valued at \$12 billion for 1983 (Parker *et al.* 1987). The majority of the pollination of this species is carried out by the solitary bee *Megachile rotundata* (Megachilidae), and so even relatively small

increases in yield due to better pollination are multiplied up to be of great importance. This bee is managed extensively using large boards with pre-existing holes drilled in it, which have been developed to provide as ideal as possible nesting sites (Torchio 1987).

Several species of ground nesting bees have been investigated for use in agriculture. Probably the most intensely studied is *Nomia melanderi*, which is also a pollinator of alfalfa. Bohart (1972) reviews in detail the techniques for establishing, transplanting and managing this species as a sustainable resource for pollination.

At the 1992 'International Workshop on Non-Apis Bees and Their Role as Crop Pollinators' at least 14 different species were presented as showing great potential for managed pollinators of commercial crops. These included several *Osmia*, *Megachile* and *Xylocopa* species. The assessment of these bees has relied on basic biological studies to establish various aspects of their nesting, foraging and parasite biology.

In order to achieve the most effective pollination system with an introduced crop or bee species, it is necessary that the crop and the bees' requirements are closely matched. This is an extremely complex business as it involves a comprehensive understanding of the biology of both the flower and the pollinator, and is entirely beyond the scope of this study. However some of the main factors that influence activity in *H. rubicundus* also influence the more general interactions of pollinator with a particular flower species, in a particular environment, and these are briefly discussed below.

Microclimate influences the behavioural patterns of all insects, and this was clearly demonstrated for *H. rubicundus* (chapter 4). If the microclimatic and

activity relations of a candidate species are known, and the microclimatological conditions in and around the crop site are also known, then it is a simple matter to eliminate any unsuitable candidates. Knowing the changes in microclimatic conditions through the day and across the season is the first step; this will allow predictions to be made as to the times at which a bee might fly by using a model based on T_a and L or T_{so} (section 4.4). The maximum and MTFF are also useful in deciding whether the flight windows of a species will coincide with the times of pollen availability. If the MTFF is exceeded for many hours through the day then it is unlikely that T_a and L will be restrictive. As common sense would predict, a bee species that is introduced to an area of similar climate to its native site is unlikely to be rendered unsuitable as a pollinator due to thermal constraints. Using a standardised system of globe temperatures to map out the microclimatic windows of a species would be a useful step forward in producing a species specific index of potential pollinators (Corbet *et al.* 1993).

The nest-site requirements of a solitary bee are also of great importance when introducing a new species, and here the findings of this thesis are particularly pertinent. For ground nesting bees, the sort of characteristics of the nest-site examined in chapter 5 would be important in determining the type of substrate that should be provided. The exact requirements will be species specific (see section 1.2), and may also encourage other hymenopteran species to nest. Introduced populations of hypogeous bees require less maintenance than do those species (e.g. *Megachile rotundata*) that are encouraged to nest in bee boards (Parker *et al.* 1987). Additionally they can form massive self-perpetuating aggregations over a period of years, and this will insure a high local abundance of suitable pollinators. Sites containing *Nomia melanderi* have been successfully managed for over 35 years in the US (Torchio 1987). The relatively short lifespan of solitary bees has allowed many species to become specialised in their foraging habits (Parker *et al.* 1987). Consequently any marked preference for a particular plant

taxon is usually associated with some sort of behavioural and/or physiological adaptation by a bee to that flower type that makes it an efficient pollinator. This can then be exploited in managed populations to ensure that the activity of a particular bee species is concentrated on the desired crop plant.

With the management of ground nesting bees there are certain problems, such as synchronising the emergence of adults with the crop bloom, and moving and transplanting the soil cores containing nests (Bohart 1972). Furthermore some species are multivoltine or partially bivoltine and this may cause problems with crops that have relatively short flower seasons. In these cases alternative forage resources must be provided outside the flowering season of the main crop; indeed, it is questionable whether a species with such a long flight season is the most suitable pollinator available.

In addition to understanding the microclimatic and nest-site requirements of a given bee species, various other important factors need to be considered before a candidate species can be proposed as a suitable pollinator. At the forage site these include: the quantity and quality of pollen and nectar rewards through time (see below); the diurnal foraging patterns in relation to floral dehiscence and stigmatic receptivity (Willmer *et al.* 1994); flight patterns and flight distances (Bataw, 1995); the effectiveness of pollen carriage and transfer leading to correct pollen-tube growth (Willmer *et al.* 1994); and competition from other nectarivores. The local predator and parasite community at the nest-site will also have effects upon the reproductive success of introduced species (section 3.7).

The problem a native bee may have with incompatible floral resources from an introduced crop are highlighted in the following example. A commercially produced variety of yellow passionfruit (*Passiflora edulis*) has been introduced to the West Indies, and is pollinated by the indigenous bee *Xylocopa mordax*. This bee

species readily collects nectar from this crop and in the process pollinates it; however, this pollen species is rejected (discarded) at the nest, and only pollen from *Gliricidia sepium* is used to form the bee loaf (Corbet & Willmer 1980). This crop then provides suitable nectar for *Xylocopa mordax*, but pollen from another source is preferred. If an alternative pollen source were not available (for instance in a large monoculture) then one might speculate that the reproductive success of this bee species might be seriously impaired through the use of poorer quality pollen; and so the overall match between bee and flower species would be poor.

Additionally this study showed that the nectar collected was less concentrated than was desired. Before storing the nectar, females evaporated water from the nectar on their tongues until the concentration was increased from 45-50 % to 62-63 %. The incidence of sub-optimal nectar quality is also demonstrated in the study of the pollination of lowland coffee (*Coffea canephora*) by *Creightonella frontalis* in Papua New Guinea (Willmer & Stone 1989). This bee visited the coffee crop to collect pollen but used other flower species as the main source of nectar. The nectar carried by *Creightonella frontalis* was considerably more concentrated than that available at the coffee. It was suggested that more concentrated nectar is required to form pollen balls that minimise the risk of fungal infection; thus in the absence of an alternate nectar source, the fitness of bees would be reduced.

Both of the above studies show the importance of matching the requirements of the pollinator with the floral resources provided by a crop. It was suggested in both cases that the effectiveness of pollination would be improved by the provision of suitable nesting sites, and also by providing alternate sources of pollen (in the passionfruit study) and nectar (in the coffee study).

At Invergowrie the incidence of *H. rubicundus* on several different varieties of raspberry was very low (Willmer *et al.* 1994), and it has been suggested that the

concentration and/or composition of the nectar may have been unsuitable for this species (Bataw 1995). Similarly in Quebec de Oliveira *et al.* (1983) found that raspberries were predominantly pollinated by *Apis mellifera* even though several species of Halictidae were found to be locally important pollinators of other flowering species. It appears then that raspberry does not provide the desired quantity and/or quality of pollen and/or nectar for *H. rubicundus*, and consequently it would be a badly matched bee-flower system.

In contrast *H. rubicundus* may be an effective pollinator of *B. napus* (section 3.4). Without further study at the floral resource sites available around Invergowrie, it is impossible to tell whether *H. rubicundus* collects nectar in addition to pollen from *B. napus*. Nectar collection might be inferred from the foraging behaviour observed in another halictid. A *Dialictus* sp. in Brazil made up $\approx 7\%$ of the total insect visitors to *B. napus* flowers, and was the third most common visitor after *A. mellifera* and *Trigona spinipes* (Adegas & Nogueira Couto 1992). This species collected both pollen and nectar while foraging (average visit times of 63 ± 44 s and 8 ± 10 s respectively); and in general it was concluded that bees are important vectors for pollen transfer on this flowering plant, and are undoubtedly of great economic benefit. The sugar concentration of oil-seed rape varies between 32-49% and is highly dependent upon microclimatic conditions (Williams 1985); the volumes and concentrations of nectar around Invergowrie need measuring directly however.

At Rothamsted Experimental Station farm (UK), it was clearly demonstrated that the yield of seeds from plots of *B. napus* varied with different pollination conditions (Williams *et al.* 1987). The mean weight of seeds per plot and mean number of seeds per plant were greater for the 'open' pollination treatment than for 'honeybee only' pollination treatment (both these were greater than for plots where insects were totally excluded). However, the pollinator species

composition of the 'open' pollinated plot is not given, and it might be that solitary bees were important pollinating agents. In the future, if honeybees are not available as pollinators (see above), then other species, including halictids, will become vital in maintaining high yields from *B. napus* and other crops.

Halictus farinosus is a common native ground nesting bee in northern Utah, and is an effective pollinator of commercial onion crops (Parker 1982). There has been limited success in transplanting this species, with few nests surviving, although techniques are considerably less developed than for other species such as *N. melanderi* (Parker *et al.* 1987). From the literature it appears that the potential of *H. rubicundus* as a managed pollinator has never been tested. It may have possibilities in improving the seed yield of *B. napus* crops; but this would need to be tested directly before any conclusion could be drawn (see section 6.6).

Overall, a knowledge of the nesting requirements and microclimatic restrictions on foraging behaviour of a bee species, in conjunction with data on floral visits, are necessary when considering the potential usefulness of an introduced pollinator. The use of managed populations of solitary bees to pollinate crops is a multi-million dollar industry (increasing yearly), and "the development of managed populations of bees as sustainable crop pollinators has always been initiated with fundamental biological studies" (Torchio 1991).

6.5 Some aspects of sociality within the Halictidae

As previously discussed, the range of sociality displayed within the Halictidae is enormous, and greater than that of any other family within the Apoidea; and variation within a single species is also well known (section 1.3).

At Invergowrie the nest cycle of *H. rubicundus* is purely solitary, with a single brood being produced which contains both males and females (section 3.2). The females are quickly mated and leave the nest-site to overwinter before returning to found a new nest the following spring and thus continue the cycle. The population of *H. rubicundus* studied by Yanega (1988), exhibited a mixture of solitary and eusocial nests. The season would commence as for the Invergowrie population, with a single fertilised female excavating a nest and providing provisions for the brood to develop. After this though, the lifestyle was clearly different; emergence occurred in June and comprised 5 to 8 individuals on average ($\approx 25\%$ males and $\approx 75\%$ females) and was protogynous (Yanega 1988). Usually three or four females would remain in the nest as workers, with the remainder mating and leaving to diapause (gynes). It is proposed that prompt mating of a newly emerged female produces a gyne, and those that are not mated quickly become workers (Yanega 1992).

If the brood was relatively small, then by chance, all the females might leave to overwinter thus effectively leaving the foundress to continue an essentially solitary lifestyle (Yanega 1988). If, however, any females remained in the nest and functioned as a worker caste by aiding the foundress (queen) in extending the nest and collecting provisions, then the nest would become primitively eusocial (Yanega 1988). Most workers were eventually mated and might even have contributed directly to the next generation by laying their own eggs in addition to those from the foundress. If the queen died soon after the first brood began to emerge then a mated worker (usually the largest) would become a replacement queen and the nest would then become an all sister (parasocial) colony. Thus the variation in social structure of Yanega's population was stochastic in nature. Eusocial nests were usual; with solitary and parasocial nests resulting (by chance) from all of first brood females dispersing, or the queen being lost, respectively.

In eusocial nests the second brood emerged in late July and was slightly smaller in number than the first. It contained $\approx 60\%$ males, with all the females being promptly mated and leaving the site to overwinter. The remaining queen and workers died off by the end of August.

Although not actually stated, the estimated average number of offspring in a eusocial nest must be greater than 12 individuals in the US (calculated from Yanega 1989), as opposed to less than five (adjusted for parasitic loss) at Invergowrie. Both populations share the same length of flight season (April to September), and as New York ($40^{\circ} 43' \text{ N}$) is in a much warmer climatic region than Scotland ($56^{\circ} 27' \text{ N}$) (Peters 1989), it is assumed that *H. rubicundus* is able to forage on more days and for longer there. In Invergowrie, a gyne will be unable to produce enough offspring quickly enough for a two brood eusocial nest to develop (see below). However, in southern UK populations eusociality has been reported (section 3.2), and it might be expected that the social structure and productivity of these nests would be similar to those of Yanega's population. In New York, there was no difference between the productivities of eusocial and parasocial nests, as both produced a mean of six offspring in the second generation.

Variation in social structure between populations has also been attributed to environmental changes which are thought to influence the proportion of males produced (Yanega 1992).^(10, 68) In temperate halictines, sex ratio appears to be dependent upon photoperiod and T_a ; with the first brood becoming more male biased as photoperiod increases (Yanega 1993). A high proportion of males will result in most females being mated promptly (and thus leaving the nest-site to overwinter); thus there will be a reduced number of social colonies overall. If females are unmated then they will remain as a worker caste and a primitively eusocial status will be attained. Ultimately, then, the latitude and time of year

during which the first brood is produced will determine the demography of a population and the subsequent social structure of the nests within it. This is consistent with the variation within the population at New York, with the sex ratio directly determining the amount of worker recruitment occurring.

In northern populations of *H. rubicundus*, such as Invergowrie, the reproductive seasons starts relatively late in the 'photoperiodic' year, owing to thermal restrictions; and so when the first brood is being laid the photoperiod is already long and the resulting sex ratio has a high proportion of males. Emergent females are all mated and become gynes; thus the population contains only solitary nests. A similar situation exists for a Colorado population (higher latitude than New York), which is also solitary (Yanega 1993). In contrast Yanega's New York population are able to become eusocial as the season is longer, and the photoperiod relatively short during the first brood.

The photoperiodic dependency of sociality is a mechanism by which social structure can be 'fine tuned' to local environmental conditions. This flexibility will have selective advantages in that it allows foundresses to respond to seasonal changes at a given location; furthermore this will aid colonisation of other sites with different environmental characteristics. This sort of environmental-dependent social plasticity has been observed in other halictids (e.g. *Halictus ligatus*, Michener & Bennett 1977; *Augochlorella striata*, Packer *et al.* 1989b). In the second study the population of *A. striata* was near the northern limit of its geographic range and was usually solitary. However, during an unusually warm season, foundresses of a small proportion of nests managed to take advantage of the extra foraging time available to initiate eusocial nests which were more than twice as productive as their solitary counterparts. At this same site in Nova Scotia, two other eusocial halictids showed very different nest productivities in response to variable weather conditions. *Lasioglossum laevis* displayed a

flexible social organisation and had a productivity of 25, whereas the inflexible and less adaptable system of *Lasioglossum cinctipes* meant that no offspring were produced in this season with unusual weather.

In addition to the productivity advantages of eusociality outlined above, there are also benefits in decreasing brood mortality due to parasites. Parasite pressure has been invoked as one of the major factors favouring the evolution of sociality in insects (Lin & Michener 1972) and in particular halictine bees (Lin 1964). As many bees nest in dense aggregations, the impact of parasites may be greater than if they were to nest in isolation when parasitism is DDD (Michener 1974). Parasite pressure in halictines may be unusually high as a result of the relatively conspicuous nature of the nest entrances; this has been suggested as one of the main reasons for the frequent origin of sociality in this group (Lin & Michener 1972). Certainly eusocial nests suffer less brood mortality than do the equivalent solitary nests. Nest guarding is found in many species of eusocial halictids (Michener 1974) and can be very effective in preventing parasite entry (e.g. *Lasioglossum zephyrum*, Batra 1965): The presence of more than a single bee within a nest may provide protection against intruders, even without specific guarding behaviour (Lin & Michener 1972; Willmer 1985, but see Field & Foster 1995). The advantages of multiple foundress associations in nest defence have been shown for *H. ligatus* (Packer 1986b), and this provides evidence for the parasocial route to sociality (reviewed by Brockmann 1984). The incidence of cell parasitism in *Lasioglossum figueresi* nests was only $\approx 5\%$ when social, and $\approx 20\%$ when solitary (Wcislo *et al.* 1993). However these data were from two different localities, and the difference could also be attributed to other factors such as lengthened developmental times experienced at the site with the solitary population.

Nest guarding was not found at Invergowrie, although parasitic Diptera were often inhibited from entering the nest when a foundress was present. It may be

that the apparent absence of this parasite from the southern UK populations is due to the eusocial status of these nests. Presumably the parasite population could never reach such high proportions as at Invergowrie as nest cells are seldom made available, because one or more females will usually be present within the nest. Eusocial behaviour will be selected for, under suitable microclimatic conditions, if it prevents the massive brood mortality experienced at sites such as Invergowrie.

Facultative sociality in halictine bees can clearly be of great adaptive significance, especially in a changeable environment. The increases in productivity arising from the recruitment of a worker caste are the result of improvements in foraging efficiency and nest defence. The advantages of this phenotypic plasticity may be explained as a facultative response of an 'all-purpose genotype' to a variable environment (Yanega 1993). The high diversity in social organisation, and existence of social plasticity, within the Halictidae may have led this group down a unique evolutionary path (Yanega 1992).

6.6 Possible directions for future studies

The Halictidae encompass an amazing variety of social behaviours, and being distributed world-wide utilise habitats with vicissitude. There are numerous opportunities for the study of the influence of environmental factors on social organisation, nesting strategies and foraging behaviour, across both populations and species.

Under the general heading of thermal biology, several questions are still to be addressed. Does *H. rubicundus* or any other temperate halictid have substantial endothermic ability? The Halictidae certainly cover the 30 - 40 mg size range previously discussed, and the family has species adapted to a broad range of thermal regimes. Individuals from more northerly populations of *H. rubicundus*

are larger and therefore may be more likely to be capable of endothermy. In addition the whole question of the applicability of Bergmann's rule at higher taxonomic levels could be tested within the Halictidae; and the abundant information on halictid ecology would help resolve any size gradients not explained by latitude.

The use of standardised T_{so} s could be further investigated to produce an index of the conditions under which various activities are observed, and this could also be used to make comparisons with other species. This will provide useful information when assessing candidate species for introduction as pollinators.

An investigation of the foraging behaviour of *H. rubicundus* at *B. napus* and other flowering plants would complement the detailed knowledge of nesting behaviour at Invergowrie. Particular attention to determining the pollen and nectar sources for this species, and other halictines, would also be of benefit in deciding which crops might be suitably pollinated by these bees.

The provision of the 'ideal nesting site' for *H. rubicundus* in an area rich with *B. napus* would present an ideal long term study in the founding, growth and decline of a nesting aggregation. Transplantation of soil cores containing overwintering females or the introduction of a number of searching foundresses to a fresh site could be undertaken to investigate the possibility of managing this species. Similar experiments could be carried out to investigate the potential usefulness of other ground-nesting bees.

Estimates of the number of bees required to pollinate a unit of crop area effectively is largely unknown for most species. Ultimately an economic analysis of the costs in managing solitary bee populations and the resultant increase in crop yield (and

therefore crop value) is desirable. Considerations of how parasites may be controlled in managed populations would also be useful.

Comparative studies of the spatial distribution of nests within aggregations would allow a better understanding of the factors that determine nest spacing. Of particular interest would be the possible influences of conspecific aggression, selfish herding, density dependent parasitism and co-operative defence.

The variation in the social organisation of *H. rubicundus* both within the UK and across Eurasia deserves further study. Specifically, in Britain it would be interesting to look at southern populations of this species and examine the social structure, nest productivity, parasite impact and the relationship of these various factors with (micro)climate. Does the same form of social plasticity exist in the UK populations as reported for North American species?

Theoretical considerations of the effect of sociality and social evolution on genetic variation have been addressed at some length. However, there is a general lack of empirical data, especially for primitively eusocial species. Some studies have begun to collect such data but it is too early for any general conclusions to be drawn. The Halictidae present the ideal group for such a study as the family contains a diverse range of social systems. Packer & Owen (1989b) have examined allozyme variation in several populations of *H. rubicundus* and several other authors are turning their attention to the Halictidae. Much of the current knowledge of the variation in sociality, behaviour and morphology in halictid populations could be explained by either phenotypic plasticity or by actual genetic differences; any studies addressing this in the near future are likely to produce important findings.

Appendix 1.

Calculation of heat transfer between *H. rubicundus* and the environment

Some very general calculations can be performed to give an indication of the relative importance of various avenues of heat exchange. The aim is to establish the approximate importance of various heat transfer mechanisms for males and females while flying and basking. Several assumptions and approximations have been necessary to carry out this procedure and the validity of these is discussed. For this kind of comparative purpose most of these assumptions will be of little importance as no attempt is made to extrapolate the findings beyond a male and female *H. rubicundus* while either basking or flying. Data are used for a single male and female bee of mean size for that sex. All the calculations are based on the thorax alone, as this is the part of the bee which is most influenced by temperature. All the measurements of body temperature were T_{th} s in this study. It is assumed that the rate of heat flow between the thorax and the head and abdomen is small and unchanged between activities. The connections between these three structures are small, but the rate of haemolymph flow can certainly be controlled in several bee species (Heinrich 1993). However these are large and endothermic species, where this kind of physiological thermoregulation will be of greater importance than for a small and ectothermic bee. Thoracic mass for mean male ≈ 4.9 mg and for female ≈ 9.1 mg; and the surface areas of thorax 7.5 and 10.7 mm² respectively (section 4.2.1).

A 1. Heat transfer while basking

A 1.1 Radiative heat exchange

The following procedure is based on the method used by Leyton (1975) for calculating the net radiation flow of a flat surface above the ground in sunshine. Dealing with each avenue of radiative heat transfer in Figure 4.1 it is possible to give an approximate value for each component. A value of 900 W m^{-2} is chosen for the level of solar radiation from the sun.

1. Direct SW radiation gained. This will be the product of the solar radiation and the absorption coefficient of the bee (α) which is $\approx 93 \%$ for each sex (section 4.2.1):

$$900 \times 0.93 = 837 \text{ W m}^{-2}$$

2. Reflected SW radiation gained. Soil has a mean absorption coefficient of $\approx 10 \%$ therefore 90% will be reflected and of this 93% will be absorbed by the bee:

$$(900 \times 0.90) \times 0.93 = 753 \text{ W m}^{-2}$$

3. LW radiation gained from the sky. This can be approximated as ' $208 + (6 \cdot T_a)$ ' from equation 9.4 in Leyton. T_a is taken as $\approx 20^\circ \text{C}$ which is the mean T_a while basking (table 4.9) and this gives a value which is then multiplied by α :

$$(208 + (6 \times 20)) \times 0.93 = 305 \text{ W m}^{-2}$$

4. LW re-radiated radiation gained from the ground. This can be approximated as ' $315 + (6 \cdot T_g)$ ' from equation 9.4 in Leyton. T_g is taken as $\approx 35^\circ \text{C}$ (associated

with a T_a of 20 °C in Figure 4.7a) and this gives a value which is then multiplied by α :

$$(315 + (5 \times 35)) \times 0.93 = 456 \text{ W m}^{-2}$$

Note: for components 1 - 4.

The SW radiation is directed from above and so half the surface area of the thorax will be assumed to absorb this (thus ignoring Lambert's cosine law); consequently this will be an over-estimate of the actual value. Likewise LW is directed from below and so absorbed by half of the surface area.

5. LW radiation lost from the bee. This can be approximated as ' $315 + (6 \cdot T_{ex})$ ' from equation 9.4 in Leyton. T_{ex} is taken as ≈ 6 °C for males and ≈ 4.5 °C for females; and this gives values which are then multiplied by α :

$$\text{males:} \quad (315 + (5 \times 6)) \times 0.93 = 320 \text{ W m}^{-2}$$

$$\text{females:} \quad (315 + (5 \times 4.5)) \times 0.93 = 314 \text{ W m}^{-2}$$

These values will apply to the whole surface area of the thorax.

Calculations 1 to 5 can be used to give approximated absolute values of radiation gain (+) and loss (-) by making them specific for surface area (all values in mW):

	Male	Female
SW solar	+3.1	+4.5
Reflected SW	+2.8	+4.0
Sky LW	+1.1	+1.6
Ground LW	+1.2	+1.7
LW from bee	-2.4	-3.4
Net SW	+5.9	+8.5
Net LW	-0.1	-0.1
Net total	+5.8	+8.4

Overall the net SW radiation gain is much more important than the LW radiation gain for both sexes, and female net radiation gain is greater than that of males.

A 1.2 Metabolic heat production

The amount of metabolic heat produced while resting was estimated from the graph of O₂ consumption against body mass (Figure 4.9, Withers 1992). The equation describing the line for ectotherms (including several insect groups) was: $y = 14.8x^{0.88}$, and when applied to *H rubicundus* gave a total O₂ consumption of 0.6 µl h⁻¹ for males and 0.61 µl h⁻¹ for females. This is equivalent to a metabolic power output of 0.36 mW for males and 0.61 mW for females (1 ml O₂ is equivalent to 21.4 J). Due to the inefficiency of muscular work 80 % of this energy is given out as heat; therefore the thermal power outputs for males is 0.29 mW and for females 0.49 mW.

A 1.3 Net heat balance

Radiative and metabolic heat gain must be balanced by convective heat loss when T_{th} remains constant; and so convection can be estimated.

	Males	Females
Radiative	+5.8	+8.4
Metabolic	+0.3	+0.5
Convective	-6.1	-8.9

A 2. Heat transfer while flying

Radiative heat gain will be reduced due to less effective body orientation to the sun and also because of the screening effect of the wings. However these values will also be modified by the different T_{ex} s of males ($\approx 5^{\circ}\text{C}$) and females ($\approx 8^{\circ}\text{C}$) during flight (section 4.5.4). The net radiation gain remains the same for males at 5.8 mW and decreases slightly for females to 8.3 mW for females.

To calculate the metabolic heat generated during flight *H. rubicundus* was assumed to be equivalent to *Anthophora plumipes* in terms of mass specific rates of power generation (Stone 1989). These rates do, however, vary between different bee taxa but the extent and direction of the differences between assumed and real values are difficult to assess (G N Stone, pers. comm.). Without further investigation it is impossible to determine whether power generation is equivalent for these two species; for the purposes of this calculation it assumed that the differences are negligible. Using a T_a of 20°C , males ($T_{ex} = 5^{\circ}\text{C}$) are estimated to generate 0.55 W g^{-1} and females ($T_{ex} = 8^{\circ}\text{C}$) estimated to generate 0.70 W g^{-1}

(from Stone 1989: Figure 4.25). For a 4.9 mg male this equates to 2.7 mW of metabolic heat and for a 9.1 mg female 6.4 mW.

The net heat balance for flying individuals (with convection calculated) is as follows:

	Males	Females
Radiative	+5.8	+8.3
Metabolic	+2.7	+6.4
Convective	-8.5	-14.7

Overall it is proposed that radiative heat loss is reduced relatively little between the basking and flying conditions. Metabolic heat is of little importance when a bee is basking but is of similar importance to radiative heat gain once flight is initiated. Metabolic heat output increases 9 fold for males and 13 fold for females between basking and flying. Metabolism may make up a much larger proportion of the total heat gain when ambient conditions are cooler and especially when L is low (providing $T_a > MTFF$); however, this relationship is not simple as power generated by the thorax also decreases as both T_a and T_{th} decrease. The influence of T_{th} , T_a and mass on power generation in *A. plumipes* are discussed fully in Stone (1989 & 1993).

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